

Seroprevalence of human cystic echinococcosis in individuals occupationally exposed to Canidae in Central Iran: A case-control study

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ABSTRACT

Echinococcus granulosus is a widespread zoonotic tapeworm that causes human cystic echinococcosis. Human CE, transmitted via dogs or Canidae feces, poses a public health challenge and exemplifies the One Health Theory. This survey was conducted to determine the seroprevalence of CE in individuals occupationally exposed (IOE) to dogs, livestock wool, and contaminated soil due to egg shedding from dogs/Canidae in industrial slaughterhouses and livestock fields, compared to a healthy control group in Isfahan province, central Iran. In a case-control study, 401 sera from IOE, including slaughterhouse workers, animal husbandry unit workers, wool industry workers, farm workers, livestock farmers, butchers, and veterinarians in the case group, were matched with 401 archived samples from the general population. All 802 samples were tested for echinococcosis IgG using ELISA. Out of 802 sera, 7 (0.9 %) tested positive for *Echinococcus* IgG. The seroprevalence in the IOE and control groups was 1.2 % (5/401) and 0.5 % (2/401), respectively. Although there was a 2.5-fold estimated risk of CE in IOE compared to the control group, this was not statistically significant. Based on the current study's findings, the overall seroprevalence of CE in the Isfahan area is similar to that of other regions in Iran.

1. Introduction

Cystic echinococcosis (CE) is one of the neglected tropical diseases (NTDs), an umbrella term recognized by the WHO and considered a public health problem in low- and middle-income countries, encompassing 20 diseases and disease groups; however, individuals living in high-income countries can also be affected (Harada et al., 2024). The life cycle of *Echinococcus granulosus* involves carnivores, particularly Canidae, as definitive hosts, while livestock and wild herbivorous/omnivorous animals serve as intermediate hosts. Ingestion of infected offal or predation may provide the definitive hosts with hydatid cysts containing numerous protoscolices, which develop into adult *E. granulosus* worms in their intestines. The hydatid cyst develops in the intermediate hosts when eggs shed in

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the feces of infected definitive hosts are ingested. Humans can also act as aberrant dead-end intermediate hosts if they accidentally ingest the tapeworm eggs, leading to CE (Alvi and Alsayeqh, 2022).

In endemic areas, the prevalence of human cystic echinococcosis is estimated to be between 1 % and 7 %, with approximately 188,000 new cases reported annually. Since 2020, human cystic echinococcosis has been included among the seven zoonotic neglected tropical diseases prioritized by the WHO, aiming to reduce the disease burden by 2030 (Aregawi et al., 2024). Cystic echinococcosis develops very slowly, leading most patients to remain asymptomatic for many years. Symptoms may manifest when the cysts enlarge, become infected, or rupture, which can result in severe complications. This may involve the liver and lungs, along with non-specific symptoms such as weight loss, malaise, and reduced appetite. Some individuals may harbor multiple asymptomatic cysts without immediate clinical signs, and the rupture of cysts represents a critical event that can lead to severe allergic reactions, secondary infections, or dissemination of the parasite. The severity of complications depends on the affected organ and whether leakage or infection occurs (Collado-Aliaga et al., 2019).

Cystic echinococcosis is primarily diagnosed through imaging techniques, with serology being less common. However, imaging methods can be costly and may not be accessible in all regions (Casulli et al., 2019); thus, serology remains widely used for primary diagnosis and serves as a relatively inexpensive follow-up tool that offers valuable insights into the disease (Frey et al., 2019). Furthermore, the clinical manifestations of patients resulting from a combination of imaging, radiographic diagnostic techniques, or pathological and histopathological evaluations suggest the presence of cystic echinococcosis CE (Weber et al., 2023). Consequently, the disease incidence in Iran is reported to be between 1.3 and 3 per 100,000 people (Shahriarirad et al., 2020a).

Cystic echinococcosis is prevalent in countries with livestock breeding, particularly hyperendemic regions where cultural practices lead to slaughtering without veterinary oversight, often in unhygienic conditions (Wen et al., 2019). These regions include Iran, India, Pakistan, Afghanistan, Turkey, and Iraq, where the cycle of disease transmission is firmly established, adversely impacting both human and animal health (Andrabi et al., 2020; Hisar, 2020; Khan et al., 2020; Mirzanejad-Asl, 2019). Consequently, this exposes occupational groups involved in animal husbandry and slaughtering—such as ranchers, livestock farmers, farm workers, slaughterhouse workers, butchers, veterinary technicians, and veterinarians—to a variety of zoonotic diseases through daily interactions with numerous herding, terrier, working and hound dogs in their workplaces. Indeed, different occupations may involve varying degrees of contact with infected dogs or environments contaminated by these animals, and dog infection may also be influenced by the context,

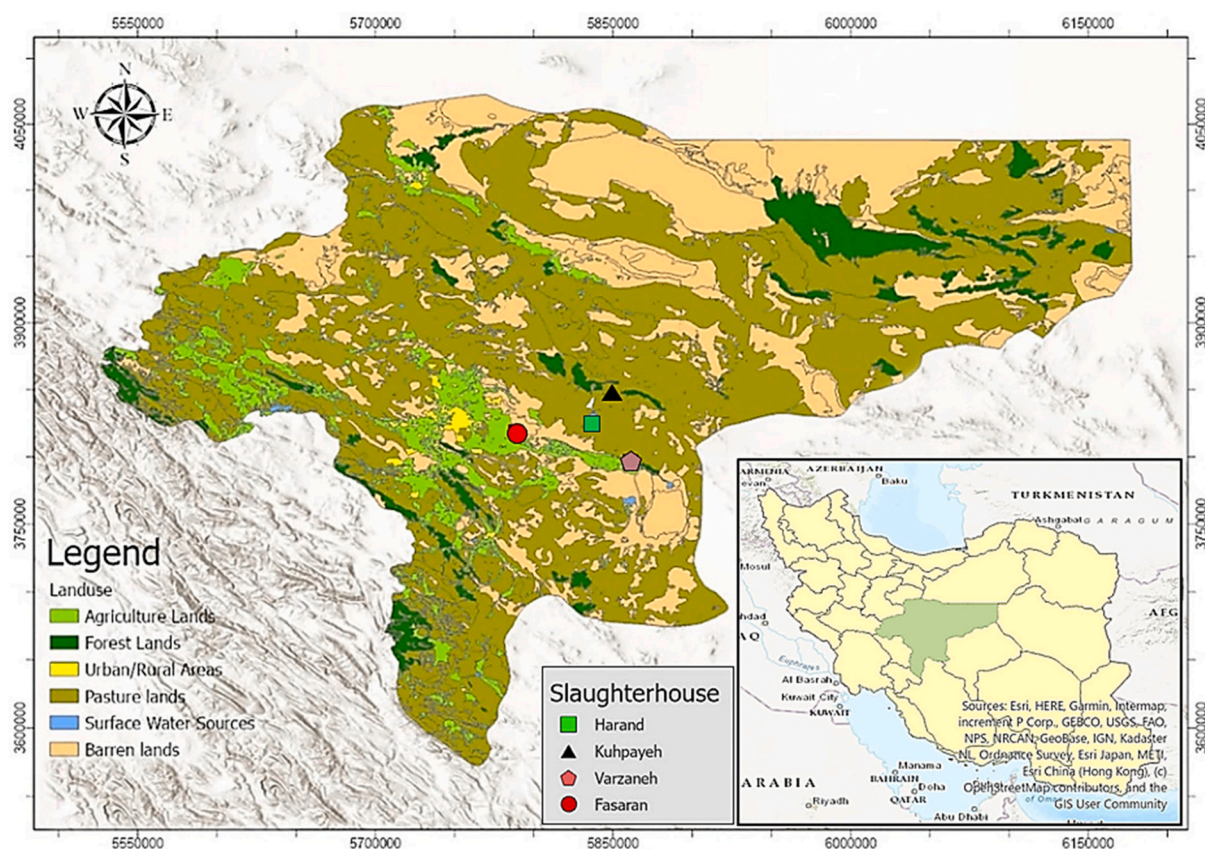


Fig. 1. The location of Isfahan province industrial slaughterhouses in which individuals occupationally exposed (IOE) to dogs/Canidae, livestock wools, and contaminated soil by eggs shedding through dogs/Canidae were investigated in this study, shown in the GIS map, which was created with QGIS software version 3.30.0 (<http://qgis.osgeo.org>).

such as whether the environment is an urban farm versus a sheep farm (Shafiei et al., 2016). The rate of cystic echinococcosis transmission among individuals occupationally exposed (IOE) in central Iran is not well established. This case-control study examined the presence of IgG antibodies against *Echinococcus* spp. in IOE and compared them with controls from the general population to clarify the occupational risk of contracting cystic echinococcosis.

2. Materials and methods

2.1. Study area

The Isfahan Province is located in the center of Iran, at approximately 32.667° N latitude and 51.917° E longitude, covering an area of about 107,018 km². Isfahan is a city that seamlessly blends its historical heritage with modern infrastructure. Surrounding the city, the rural areas consist of smaller villages primarily engaged in agriculture. The Zayandeh Rud River supports the ancient canal system and is essential for agriculture in the region. Generally, urban areas have better access to facilities, while rural regions suffer from inadequate resources. A notable number of dogs also inhabit Isfahan Province. While most urban dogs are kept as pets, many are free-ranging or stray, particularly in rural areas. In these circumstances, unowned dogs may pose health risks due to overpopulation and the potential transmission of zoonotic diseases. Four sampling slaughterhouses are illustrated in Fig. 1.

2.2. Study design and participant recruitment

A case-control study was conducted among four industrial slaughterhouses in Fasaran, Varzaneh, Harand, and Kohpayeh in 2021 (Fig. 1). The study received ethical approval under the ethics code IR.MUI.MED.REC.1399.1048. A total of 401 sera were collected from IOEs (including slaughterhouse and animal husbandry workers, wool picking workers, farm workers, livestock farmers, butchers, and veterinarians) as the case group. Participants were informed about the study and provided informed consent. Sampling was performed using simple random sampling with random number tables. Demographic data were recorded on the questionnaires after subjects consented to participate. Three milliliters of blood were drawn from each of the 401 cases, and the sera were separated and frozen at -20 °C until analysis. A control group comprising 401 sera from the general population was also examined for *Echinococcus* IgG and compared with the case group. This control group consisted of individuals referred to the provincial laboratory from various parts of Isfahan, all having occupations different from those in the case group, with attention to matching by gender and age.

2.3. ELISA test

All sera were individually tested for IgG antibodies using a commercial semi-quantitative enzyme-linked immunosorbent assay kit (*Echinococcus* IgG ELISA Kit, Pishtaz Teb, Iran). The test includes purified antigens, with a sensitivity of 91 % and specificity of 96 %, as reported by the manufacturer. Initially, both positive and negative cut-offs were loaded onto the 96-well plate. Diluted (1:100) serum samples were added to the respective wells in triplicate. After shaking for 5 min, the plate was covered and incubated at 37 °C for 60 min. The plate contents were emptied, and a washing solution was applied three times. After adding 100 µL anti-human IgG HRP conjugate, incubation at 37 °C was carried out for 60 min. The plate was then washed again, and 100 µL substrate was added to each well, incubating at room temperature in a dark area for 20 min. Finally, 50 µL stop solution was added to each well, and the absorbance was determined using the EZ Read 400®, Biochrom Microplate Reader at 450 nm.

2.4. Sample size

The required sample size was calculated based on a previous study conducted among different populations in Iran (Shahriarirad et al., 2020b) with a confidence level of 95 % ($z = 1.96$) and a margin of error of 5 % ($d = 0.05$), resulting in a calculated sample size of 338 cases in the human population. Considering a 20 % allowance for potential sample loss, the final sample size was adjusted to 400.

2.5. Data collection tool

The questionnaire used in this study consisted of three sections. The first section contained a brief introduction to the study's topic and objectives, as well as a request for consent to participate. The second section focused on socio-demographic and scholarly information, including age, gender, education level, occupation, hand hygiene practices, and duration of work. The third part assessed participants' awareness and knowledge of CE through various items that primarily covered the disease's origin, reservoirs, clinical indicators, lesions in humans and animals, diagnosis, and prevention. Participants' knowledge levels were evaluated based on whether they had previously heard of the disease.

2.6. Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 22.0. Statistical descriptions were employed, utilizing frequencies to analyze past CE knowledge and practices. The association of categorical variables was evaluated using the Chi-squared test. Binary logistic regression was employed to determine odds ratios in variables with more than two variants. Factors related to prior knowledge of cystic echinococcosis, with a significance level set at a p -value of 0.05.

3. Results

Of the 802 tested sera, 7 (0.9 %) were positive for *Echinococcus* IgG. The seroprevalence of CE in the IOE and control groups was 1.2 % (5/401) and 0.5 % (2/401), respectively. Despite the 2.5-fold higher risk of infection in the case group, Chi-square analysis revealed a non-significant difference in *Echinococcus* seroprevalence between the two groups ($p = 0.225$) (Table 1).

Among the 802 studied individuals, there was no significant difference in the mean age of *Echinococcus* IgG seropositive and seronegative individuals. Additionally, this difference was not statistically significant in the control group alone. However, in the 401 cases, the mean age of seropositive individuals was significantly higher than that of seronegative ones (Table 2).

This finding also held true when dividing individuals into age groups, where no significant difference was observed in the total population or controls, yet a significant difference was detected among cases, showing higher positivity in older age groups (Table 3).

No significant differences in specific anti-*Echinococcus* IgG antibodies were found among the studied demographic variables, including gender, education, occupation, hand hygiene, and work duration (Table 4).

4. Discussion

In the present study conducted in central Iran, the seroprevalence of CE in the general population was 0.5 % compared to 1.2 % in the studied IOE individuals. The low rate of seroprevalence in the general population, compared to higher rates reported in other studies, may be due to the selection of the study population. Most studies evaluate individuals with special conditions, but we specifically selected individuals who had jobs not in contact with the definitive host for the control group, allowing for appropriate comparisons with the case group. Our results showed that despite the risk of infection in IOE being 2.5 times that of non-exposed individuals, it was not statistically significant. Considering age, higher positivity was observed in older ages.

The high prevalence of CE was recorded in Eastern Mediterranean (EMRO) countries, such as Iran, where millions of people's livelihoods exclusively depend on livestock and human cases have been reported from nearly every province. The estimated pooled prevalence of CE in Iran from 1990 to 2015 was 3 % to 5.5 %. Moreover, unsuitable animal slaughter practices prevail in traditional areas, and dogs, as definitive hosts, are fed hydatid cyst-infected organs. On the other hand, the seasonal migration of nomadic pastoral communities with their large herds and herding dogs, along with the movement of wild animals (both prey and predators), increases the disease's expansion (Khalkhali et al., 2018).

The prevalence of hydatid cysts among rural residents who own dogs in Khuzestan province, Iran, was reported to be 4.9 %, while it was estimated to be 13.8 % among nomads (Mahmoudi et al., 2019). In a study conducted in Ahvaz and Shiraz in 2019 on urban waste collectors, positive rates for hydatid cysts were 3.7 % and 26.3 %, respectively (Erfani et al., 2020; Molouk et al., 2019). In the present study, *Echinococcus* IgG seropositivity among IOE in Isfahan, central Iran, was 1.2 %. Comparing our survey's serological results with studies from the Shiraz and Khuzestan Provinces indicates that climate and humidity may affect the persistence of infectious eggs in the environment. Compared to the mentioned regions, Isfahan province has lower humidity, and the desert climate is a significant aspect of the province. Additionally, the culture of the people in this province, along with the municipality's health performance and natural resources, stands out in the entire country of Iran, which can significantly reduce disease transmission.

Investigating the epidemiological status and clinical characteristics of hydatid cysts in patients admitted to hospitals in northern Iran over ten years showed the highest and lowest prevalence of CE in the age range of 50–59 years at 19.0 % and in individuals over 80 years at 5.0 %, respectively. The male-to-female ratio was 0.88 (47.0 % vs. 53.0 %), and the majority of cases were urban residents (54.0 %) with no close contact with animals (58.0 %) (Parkoobi et al., 2018). In the present case-control study, we observed higher seropositivity in males, and it was also higher in the IOE (case group), yet it was statistically nonsignificant compared to the general population (control group).

A descriptive-analytical study involving 360 patients from various regions of southwestern Iran diagnosed with CE over 15 years indicated that most patients were in the age group over 50 years (28.6 %) (Kamali et al., 2018). In our investigation, only the case group (IOE) showed an increased risk of infection with older age. Additionally, no significant relationship was found between gender, contact with animals, and living area (Kamali et al., 2018). In our study, no statistically significant relationship was observed between gender, education level, and infection rate. However, Mahmoudi et al. (2019) reported higher *Echinococcus* infection rates in home-makers (31 %), illiterate individuals (14 %), and farmers (12 %), likely due to increased contact with Canidae shedding *Echinococcus* spp. eggs in the environment. Contaminated soil and livestock wool by the parasite's eggs are common transmission routes to humans (28.6 %) (Mahmoudi et al., 2019).

Echinococcus granulosus adult worms have been identified in various carnivores across many provinces of Iran (Dalimi et al., 2002; Mobedi et al., 2013; Rokni, 2009). In Isfahan provinces, 27.1 % of dogs were reported to be infected (Pestehchian et al., 2012). A study in northwest Iran indicated that most dog owners are infected (Mirzanejad-Asl, 2019). In contrast, there was no statistically significant

Table 1

The p -value and Odds ratio estimated for *Echinococcus* IgG seropositivity among case (IOEs) and control groups using the Chi-square test.

Group	<i>Echinococcus</i> IgG		Odds ratio	p	Total
	Negative	Positive			
IOE No. (%)	396 (98.8)	5 (1.2)	2.5		401
Control No. (%)	399 (99.5)	2 (0.5)	1	0.255	401
Total	795 (99.1)	7 (0.9)			802

Table 2

The mean age comparison among *Echinococcus* IgG seropositive and seronegative humans using the Mann-Whitney *U* test. The distribution of age was not normal defined by one-sample Kolmogorov–Smirnov test.

Mean age in groups		N	Mean	Mean rank	p-value
All participants	Negative	795	40.22	400.14	0.077
	Positive	7	49.86	555.57	
	Total	802			
Case group	Negative	396	36.89	199.27	0.004
	Positive	5	55	337.80	
	Total	401			
Control group	Negative	399	43.53	201.33	0.426
	Positive	2	37	136.00	
	Total	401			

Table 3

The *p*-value estimated for anti-*Echinococcus* IgG seropositivity in different age groups in all 802 studied humans, 401 cases (IOE) and 401 controls (normal population).

Age Groups	All individuals		<i>p</i>	Total	Case		<i>p</i>	Total	Control		<i>p</i>	Total
	Negative	Positive			Negative	Positive			Negative	Positive		
<20	33 (100 %)	0 (0 %)	0.358	33	24 (100 %)	0 (0 %)	0.023	24	9 (100 %)	0 (0 %)	0.942	9
20–40	376 (99.5 %)	2 (0.5 %)		378	224 (99.6 %)	1 (0.4 %)		225	152 (99.3 %)	1 (0.7 %)		153
40–60	307 (99 %)	3 (1 %)		310	122 (98.4 %)	2 (1.6 %)		124	185 (99.5 %)	1 (0.5 %)		186
>60	79 (97.5 %)	2 (2.5 %)		81	26 (92.9 %)	2 (7.1 %)		28	53 (100 %)	0 (0 %)		53
Total	795 (99.1 %)	7 (0.9 %)		802	396 (98.8 %)	5 (1.2 %)		401	399 (99.5 %)	2 (0.5 %)		401

Table 4

The *p*-value and Odds ratio estimated for *Echinococcus* IgG seropositivity for different risk factors and demographic variables in 401 individuals occupationally exposed (IOE) using the Chi-square test for bivariate variables and binary regression for those with more than two variables.

Variable		<i>Echinococcus</i> IgG		Total	OR	<i>p</i>
		Negative N (%)	Positive N (%)			
Gender	Male	382 (98.7)	5 (1.3)	387	1	0.83
	Female	14 (100)	0 (0)	14	0.97	
	Illiterate	115 (99.1)	1 (0.9)	116	1	
Education	School	232 (98.7)	3 (1.3)	235	1.48	0.83
	University	49 (98)	1 (2)	50	2.34	
	Farmers/Farmworkers	286 (98.6)	4 (1.4)	290	1	
Occupation	Butchers/Livestock farmers/Animal husbandry unit workers/Picking wool workers	99 (100)	0 (0)	99	–	0.27
	Veterinarians/Slaughterhouse workers	11 (91.7)	1 (8.3)	12	6.5	
Hand hygiene	No	335 (98.8)	4 (1.2)	339	1	0.57
	Yes	61 (98.4)	1 (1.6)	62	1.37	
Work Duration	1–9	115 (100)	0 (0)	115	–	0.81
	10–19	96 (99)	1 (1)	97	0.48	
	≥20	185 (97.9)	4 (2.1)	189	1	
Total		396 (98.8 %)	5 (1.2 %)	401 (100 %)		

relationship between seropositivity in selected occupations related to IOE and Canidae, livestock wools, and contaminated soil due to eggs shed by Canidae in industrial slaughterhouses and livestock fields compared to the control group in Isfahan province, central Iran.

A firm response to CE control will require interdisciplinary and transdisciplinary partnerships (Harada et al., 2024). Given the comparable disease ecosystems, livestock trade pathways, and management systems, it is crucial to understand disease epidemiology across different regions. (Aregawi et al., 2024). Training for abattoir personnel must guarantee that they are well-versed in the latest guidelines and optimal practices for mitigating the spread of infectious diseases (Acke et al., 2022; Lounis et al., 2024).

People employed in the occupations targeted for interviews often had poor interpersonal skills, likely due to the nature of their jobs. This issue resulted in some data loss during demographic information collection. Additionally, one limitation of the study was the lack of demographic information in the control group and the non-uniformity in the number of people from different occupations included in the study; during statistical analysis, this small sample size and few positives created limitations in the evaluations. Furthermore, the use of ELISA-based serology, rather than expensive imaging methods for detecting *Echinococcus*, represented a limitation of the study.

5. Conclusion

The findings of the present study indicate that the seroprevalence of cystic echinococcosis in Isfahan is relatively low compared to other regions of the country. However, it is noteworthy that this prevalence is higher among individuals who have direct or indirect exposure to Canids, yet the difference is not statistically significant. Additionally, the results suggest that the risk of infection increases with age. Given the unique characteristics and low prevalence of this infection, it is recommended that further research be conducted with larger sample sizes to gain a more comprehensive understanding.

CRedit authorship contribution statement

Seyed Hossein Hejazi: Writing – review & editing, Validation, Resources, Methodology. **Reza Kalantari:** Writing – original draft, Data curation, Conceptualization. **Seyed Mahmoud Mousavi:** Writing – original draft, Software, Formal analysis. **Marzieh Safari:** Writing – review & editing, Software, Formal analysis. **Zahra Ghayour:** Writing – review & editing, Supervision, Funding acquisition. **Zary Nokhodian:** Writing – original draft, Supervision, Project administration. **Mahsa Esmaeilifallah:** Writing – review & editing, Writing – original draft, Investigation, Data curation.

Ethics statement

Ethical approval has been obtained from the Research Ethical Committee of Isfahan University of Medical Sciences with ethics number IR.MUI.MED.REC.1399.1048.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Seyed Hossein Hejazi reports financial support was provided by Isfahan University of Medical Sciences. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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