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Seroprevalence of Human Anti-*Echinococcus granulosus* Antibodies in Saveh, Central Iran

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ABSTRACT

Background: We aimed to investigate the seroprevalence of anti- *Echinococcus granulosus* (*E. granulosus*) antibodies in humans in Saveh, Central Iran.
Methods: A random sample of 199 participants referred to the health centers in Saveh, Markazi Province, central Iran was selected. Informed consent was obtained, and the participants completed a questionnaire. Three milliliters of venous blood were collected in the Parasitology Laboratory of Saveh University of Medical Sciences. The Pishtaz Teb ELISA kit was used to detect anti-*E. granulosus* antibodies, following the manufacturer's protocol.
Results: The seroprevalence of anti-*E. granulosus* antibodies was 6%. The prevalence was slightly higher in men (6.9%) than in women (5.1%). The 26–40 yr age group had the highest prevalence (11.1%). Most participants were freelancers, and this group exhibited the highest seroprevalence (11.4%). There was no statistically significant association between antibody presence and age, sex, occupation, or place of residence. Among the 30 participants who kept dogs, 4 tested positive. A personal or family history of hydatid cysts was not significantly associated with seropositivity. Of the 15 participants who did not wash vegetables with disinfectants, 2 were antibody-positive. Not washing hands before eating was significantly associated with infection, with 18.5% of those who did not wash their hands testing positive (P=0.001).
Conclusion: The presence of anti-*E. granulosus* antibodies in Saveh indicates ongoing exposure and an elevated risk of infection. Implementing preventive measures—such as regular monitoring and screening, health education, and reducing contact with stray dogs—is essential to control hydatidosis in this region.

Key words: *Echinococcus granulosus*, Hydatid cyst, Seroprevalence, Iran

Introduction

Hydatidosis is one of the most significant zoonotic diseases and ranks as the second most important among diseases caused by helminths (1, 2). This disease is prevalent in many parts of the world, especially in countries with thriving livestock farming, where it causes substantial economic and health losses annually (3, 4). The causative agent of hydatidosis is the tapeworm *Echinococcus granulosus*; its most important definitive host is canids, and its intermediate host is herbivores, particularly sheep (5). Humans, acting as an accidental intermediate host and a biological dead end, become infected with the parasite's eggs through the ingestion of contaminated water, vegetables, or food, and/or by touching infected dogs.

Ultimately, the ingestion of these eggs represents one of the pathogenic stages of the parasite for humans (4-6) .Hydatidosis is caused by the growth of metacestode cysts in the intermediate host. In humans, the severity of symptoms and complications depends on the extent of the infection and the location of the larval (cyst) formation in the body. In general, hydatid cysts are most often located in the liver and lungs, but other organs, such as the muscles, spleen, heart, brain, and bone marrow, can also be sites of larval (hydatid cyst) settlement (6, 7). Hydatidosis has been reported in all provinces of Iran, with Khorasan Province having the highest rate of infection in humans (4.5 per 100,000) and Hormozgan Province having the lowest rate (0.1 per 100,000). For the whole country, the average rate of surgical cases due to hydatid cysts has been estimated at 1.2 per 100,000 (8).

Over the past decades, about 10 subspecies of the *E. granulosus* species have been defined by different researchers, most of which were defined by Rausch in 1954 (9). Due to the lack of specific clinical signs and symptoms, the diagnosis of hydatid cysts relies mostly on imaging techniques (10). However, serological tests are often preferred for initial screening and diagnosis over imaging methods because they are easier to use, more cost-effective, and do not require complex equipment (11). Since rapid and accurate diagnosis is essential for disease control, treatment, and preventing recurrence, the use of immunological methods is important. Among these, immunoelectrophoresis tests, Casoni skin tests, IHA, and IFA have demonstrated lower sensitivity and specificity than ELISA and Western blot methods for detecting antibodies against *E. granulosus* (11). Furthermore, ELISA serology using Antigen B (Ag B) has been widely employed for diagnosing human hydatidosis (2, 11, 12).

This study, for the first time, aimed to investigate the seroprevalence of human hydatidosis in Saveh, Central Iran.

Materials and Methods

Ethics approval

Ethical issues were fully observed by the authors. The ethics code for this study is IR.SAVEHUMS.REC.1399.036.

Study area and sampling

The study was conducted in Saveh, Markazi Province, located in central Iran (Figure 1). Previous research indicated that the average prevalence of hydatidosis in Iran was 0.6-1.2/100000 (13). Based on this prevalence, with a 95% confidence level and a 25% margin of error, the calculated sample size was 1,168 participants. However, due to practical limitations such as laboratory capacity and the high cost of diagnostic kits, the sample size was reduced to 199 participants. Simple random sampling was performed among individuals who visited comprehensive health centers in both rural and urban areas of Saveh.

After obtaining approval from the Health Deputy of Saveh, samples were collected from con-

senting patients following the completion of a questionnaire. The questionnaire gathered personal information, including name, age, gender, occupation, place of residence, dog ownership, and history of hydatid cyst infection.

Fig. 1: Saveh, Markazi Province, located in central Iran

Serological assay

To identify and analyze the presence of anti-*E. granulosus* antibodies in a serological test, 5 ml of blood was obtained, which, following centrifugation (10 min, 500×g), yielded a separated sample of serum, which was then stored at -20 °C. In this way, daily, the serum samples were transported in special containers to the Parasitology Research Laboratory of Saveh Faculty of Medical Sciences. The ELISA test was carried out with the use of the Pishtaz Teb kit (Tehran, Iran) in accordance with the manufacturer's instructions.

Statistical analysis

SPSS version 26 (IBM Corp., Armonk, NY, USA) was used for data analysis. Associations between variables were evaluated by the Chi-square and Fisher's exact test (P<0.05). Multivariate logistic regression analysis with a 95% confidence interval was used to calculate adjusted odds ratios (ORs).

Results

Twelve out of 199 participants (6.03%) tested positive for anti-*E. granulosus* antibodies (Table 1). The prevalence was slightly higher in men (6.93%) compared to women (5.10%). The 26–40 years' age group had the highest prevalence at 11.1%, with six individuals testing positive in this category. Most participants were freelancers, among whom the prevalence was highest at 9.1%. The majority of participants (63.3%) resided in urban areas, which also showed a higher prevalence rate (6.3%) compared to rural areas. However, no statistically significant association was found between seropositivity and age, gender, occupation, or place of residence.

Of the 199 participants, 30 participants (15.1%) reported owning dogs, of whom 4 (13.3%) tested positive for anti-*E. granulosus* antibodies. The difference in antibody prevalence between dog owners and non-owners was statistically significant (P=0.001). Among all participants, only two reported a personal history of hydatid cysts, and both (100%) were seropositive. Conversely, three participants reported a family history of hydatid cysts, all of whom tested negative for the antibodies (100%). However, nei-

ther personal history (P=0.493) nor family history (*P*=0.348) of hydatid cysts was significantly associated with seroprevalence. Fifteen participants reported not washing vegetables with disinfectants before consumption. Among this group, 2 tested positive, demonstrating a statistically significant association between lack of disinfectant washing and infection risk. Furthermore, not washing hands before eating was associated with a higher seropositivity and showing a significant correlation with infection (Table 1).

The results from the multiple multinomial logistic regressions revealed that the seroprevalence of anti-*E. granulosus* IgG antibodies in subjects who did not wash vegetables with disinfectant were 4.8 times higher than in individuals who did wash vegetables with disinfectant. However, there was no significant association between handwashing and *E. granulosus* IgG seropositivity in individuals who kept dogs (Table 2).

Table 1: Seroprevalence of *E. granulosus* antibodies in patients referred to comprehensive health service centers in Saveh according to different variables

Variable	***Groups***	***Total (%)***	***Positive (%)***	***P value***
Gender	Man	101 (50.8)	7 (6.9)	0.280
	Woman	98 (49.2)	5 (5.1)	
Age (yr)	<25	53 (26.6)	2 (3.7)	0.298
	26-40	54 (27.1)	6 (11.1)	
	41-60	60 (30.2)	2 (3.3)	
	>61	32 (16.1)	2 (6.3)	
Job	Freelance	55 (29.6)	5 (9.1)	0.765
	Retired	18 (9.0)	1 (5.5)	
	Housewife	49 (24.6)	3 (6.1)	
	Student	16 (8.0)	0 (0)	
	Employee	46 (23.1)	2 (4.3)	
	Under high school	15 (5.5)	1 (6.7)	
Residence	Urban	126 (63.3)	8 (6.3)	0.620
	Rural	73 (36.7)	4 (5.4)	
Keeping a dog at home	Yes	30 (15.1)	4 (13.3)	0.001*
	No	169 (84.9)	8 (4.7)	
Previous History of hydatid cyst	Yes	2 (1)	2 (100)	0.493
	No	197 (99)	10 (5.1)	
Family history of hydatid cyst	Yes	3 (1.5)	0 (0)	0.384
	No	196 (98.5)	12 (6.1)	
Washing vegetables with disinfectant	Yes	184 (92.5)	10 (5.4)	0.022*
	No	15 (7.5)	2 (13.3)	
Washing hands before eating	Yes	167 (83.9)	6 (3.6)	0.001*
	No	32 (16.1)	6 (18.7)	
Total		199 (100)	6.0)	-

*Significant at the level of 0.05

Table 2: Multiple logistic regression analysis for seropositive subjects in health centers in Saveh

Risk factors	*Odds ratio (95% CI)*	*P value*
Keeping dog at home	1.8 (0.33 , 10.1)	0.001*
Washing vegetables	4.8 (0.26 , 16.2)	0.022*
Washing hands	1.2 (0.13 , 11.1)	0.001*

Discussion

In the present study, the seroprevalence of human hydatid cysts in Saveh was reported to be 6% for the first time. Previous studies conducted in Ardabil (4.4%), Yasuj (7.2%), Fars (8.73%), Kerman (7.3%), and Chaharmahal va Bakhtiari (4.8) in Iran reported different figures (14-18). This prevalence was also different from the results of studies conducted in Mazandaran (31.6%), North Khorasan (3.96%), Shahr-e Babak city (0.18), and Arak (3.46%) (19-22). Climate can be a very important factor in determining the prevalence of this parasite in different regions (23). On the other hand, parasite eggs are more sensitive to high temperatures and low humidity, and they are destroyed after a short period of time, which can lead to a lower prevalence of hydatid cysts in relatively warm and dry regions (24).

The seroprevalence rate in men was higher than in women in the present study, which is consistent with the findings of other studies (18)). However, this study observed no significant relationship between gender and seropositivity to the hydatid cyst. Conversely, a study conducted in Bandar Abbas Province, Southern Iran, reported a higher prevalence of hydatid cyst antibodies in women. This finding was attributed to women's greater contact with vegetables and other foods contaminated with parasite eggs (25).

Age was another factor examined in this study. The results showed that the 26 to 40 yr age range had the highest number of positive cases of hydatid cyst antibodies (11.1%), consistent with studies conducted in Golestan and Arak in Iran (19, 26). On the other hand, Shafiee et al. identified the 21-30 year age group as having the most individuals infected with hydatid cyst antibodies (22). Age can be an important factor in the seroprevalence of hydatid cysts, and this prevalence tends to vary in different populations. Young to middle-aged individuals, due to their peak work activity, may be prone to a higher prevalence of positive cases (27).

Other variables examined in this study, including keeping dogs indoors, washing vegetables before consumption, and washing hands before eating, showed a significant association with the prevalence of positive seroprevalence for hydatid cysts (P<0.005). These results were consistent with studies conducted in Hamedan and Mazandaran provinces (20, 28), while they differed from those conducted in Kerman and North Khorasan provinces (15, 22). Direct or indirect contact with animals has been stated in many studies as a major factor in the spread of the disease in both endemic and non-endemic areas. Furthermore, vegetables consumed in the country represent another problem for the spread of hydatid cysts (29), due to the methods of cultivation and production and the high number of stray dogs in cultivation areas.

Fifteen participants (7.5% of the sample) reported not using disinfectants to wash their consumed vegetables, and 2 of these individuals (13.3%) tested positive for antibodies. This result suggests a significant association between failing to use disinfectants for vegetable washing and the incidence of infection ($P = 0.022$).

Multiple logistic regression analysis revealed a significant association between *E. granulosus* IgG seropositivity and vegetable washing practices ((P<0.05). This suggests that the consumption of unwashed or improperly washed

vegetables may constitute a significant transmission route for hydatidosis within the studied population. Contamination of vegetables with parasite eggs—likely originating from soil or water exposed to dog feces—can facilitate ingestion and subsequent infection. Therefore, promoting proper hygiene, such as washing vegetables with appropriate disinfectants, could reduce human exposure to *E. granulosus*. This finding corroborates existing literature highlighting the role of environmental contamination and foodborne transmission in cystic echinococcosis epidemiology (19,25-27). It also highlights the importance of public health education focusing on food hygiene to prevent infection in endemic regions (30).

Failure to wash hands before eating caused 18.5% of people to contract the disease, and the relationship of this variable to the incidence was also statistically significant (P=0.001). Observing personal hygiene and disinfecting food before consumption not only prevents people from contracting parasitic diseases but also provides a basis for controlling and preventing many other pathogenic microorganisms in humans, a fact demonstrated in numerous studies.

While the present study provides valuable insight into population exposure to *E. granulosus*, the interpretation of serological results requires consideration of the inherent limitations of this method. The ELISA for detecting IgG antibodies to the parasite, although suitable for large-scale epidemiological studies due to its ease of use and relatively low cost, faces significant challenges in terms of sensitivity and specificity (31, 32). The sensitivity of this test is significantly affected by the location, number, and status of the cysts. For example, hepatic cysts usually elicit a stronger antibody response, whereas pulmonary, cerebral, or calcified cysts may elicit a weaker serological response or result in false negatives (33). On the other hand, the cross-reactivity of *E. granulosus* antigens with other cestode parasites, such as *Taenia solium* (the causative agent of cysticercosis), and even some fungal infections, can lead to false-positive results and reduce the test's specificity (34, 35). Therefore, the prevalence rates reported in seroepidemiological studies of hydatid cysts may combine cases of active infection, past infections, and several false positives, and thus do not necessarily equate to the true prevalence of the disease.

The efficiency and accuracy of serological testing are highly dependent on the nature and quality of the antigen used in the kit. The two main antigens used in the diagnosis of hydatidosis are Antigen B (AgB) and Antigen 5 (Ag5) (35). Antigen B (AgB), which is a sensitive lipoprotein, is often used as the antigen of choice in commercial kits (such as the kit used in this study). However, AgB is also prone to cross-reactivity. Antigen 5 (Ag5), which is a glycoprotein, has higher diagnostic specificity but may show lower sensitivity in the early stages of infection or with single cysts. The use of antigen cocktails consisting of both antigens (AgB and Ag5) or purified recombinant antigens can provide a better balance between sensitivity and specificity (36, 37). Therefore, the choice of antigen in a diagnostic kit has a direct impact on the results of epidemiological studies; consequently, comparisons between studies that have used different antigens should be made with caution. Furthermore, uncertainty regarding the exact composition of antigens in commercial kits presents an additional challenge.

Given their limitations, the ELISA-based findings of this study primarily indicate parasite exposure or population seropositivity. For a definitive diagnosis of active infection—and to differentiate it from past exposure or false positives—we propose a combined strategy: integrating serological results with imaging methods like ultrasound or CT scan (the diagnostic gold standard). This integrated approach is crucial for guiding therapeutic decisions, particularly in clinical settings. Nonetheless, serological studies, such as this one, offer a valuable, low-cost, and simple tool for initial surveil-

lance, identifying high-risk areas and populations, and forming the basis for public health intervention planning and further follow-up research.

Study limitations

Several limitations should be acknowledged when interpreting the findings of this study. Primarily, the final sample size was significantly smaller than the initially calculated requirement, a consequence of budgetary constraints. This reduction potentially compromises the precision of the prevalence estimate and diminishes the statistical power necessary to identify significant associations. Secondly, the cross-sectional design of the research design prevents the establishment of causal relationships. Thirdly, the reliance on the IgG ELISA test introduces ambiguity, as it fails to differentiate between active and past infection, with performance further modulated by the specific characteristics of the diagnostic kit utilized. Lastly, the reliance on participant self-reporting for high-risk behaviors introduces the potential for recall or social desirability bias.

Conclusion

This study demonstrated a seroprevalence of 6% for *E. granulosus* in individuals attending Comprehensive Health Service Centers in Saveh. Furthermore, the findings revealed a significant association between parasite prevalence and both contact with dogs and poor personal and dietary hygiene. Therefore, developing and implementing targeted prevention and control strategies is essential. These efforts must include controlling the stray dog population, regularly monitoring and screening livestock for hydatid cyst infection, and educating the community to raise public awareness about the parasite's life cycle and the importance of hygienic practices to reduce transmission risk

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Conflict of interest

No potential conflict of interest was reported by the authors.

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