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Original Article

A Seroepidemiological Survey on the Associated Risk Factors of Cystic Echinococcosis in South Khorasan Province, Iran

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Abstract

Background: Cystic echinococcosis (CE) is a neglected zoonosis caused by *Echinococcus granulosus*. This cross-sectional study aimed to determine the seroepidemiological status and associated risk factors of CE in South Khorasan province, Iran.

Methods: Overall, 400 participants were systematically recruited from comprehensive health service centers. Demographic data and risk profiles (dog contact, raw vegetable consumption, and handwashing routines) were collected after obtaining informed consent. Serum samples were tested for anti-*Echinococcus* immunoglobulin G using a commercial enzyme-linked immunosorbent assay (ELISA) kit. Finally, the associations between seropositivity and various exposures were assessed using chi-square tests and logistic regression.

Results: In general, 5 out of 400 subjects tested positive, yielding an overall seroprevalence of 1.25%. Although men exhibited a marginally higher rate (2.3%) than women (0.7%), this difference was not significant. Irregular handwashing and frequent consumption of raw vegetables revealed significant associations with increased seroprevalence. Meanwhile, dog ownership, urban vs. rural residence, and educational status demonstrated no statistically significant relationships. Moreover, farmer occupation had notably higher positivity (8.5%) than other groups.

Conclusion: Our findings highlight the role of personal hygiene and dietary practices in transmission. Despite a relatively low prevalence, CE remains an ongoing public health concern in Birjand, particularly for certain high-risk groups. Accordingly, preventive efforts (e.g., promoting regular handwashing, ensuring careful vegetable cleaning, and strengthening livestock management) merit attention from local health authorities.

Keywords: Hydatid cyst, Seroepidemiology, Risk factors, ELISA

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Introduction

Cystic echinococcosis (CE) caused by *Echinococcus granulosus* is globally distributed, with highest incidence rates often observed in pastoral communities that have close dog–livestock–human contact (1,2). In many developing regions (e.g., parts of South America, North Africa, Central Asia, and the Middle East), poor sanitation and widespread free-roaming dogs facilitate egg contamination of pastures and water sources (3). Globally, CE distribution has been partially shaped by climatic, socioeconomic, and cultural factors (4). In Iran, for example, a predominantly livestock-based living in certain regions contributes to elevated transmission risks (5,6). Regional variations in parasite prevalence, dog ownership, and the degree of veterinary oversight at abattoirs imply that local epidemiological data are essential for designing effective control measures (7). Additionally, the improper handling of livestock offal, close human–dog contact, and insufficient awareness of food hygiene can facilitate ongoing infection cycles (8). Prevalence estimates for CE markedly vary by location. For instance, rates can be under 1% in some urbanized areas, whereas they can exceed 10% in more rural regions. These disparities are often due to differences in husbandry practices, health infrastructure, and public awareness (9,10). The semi-arid climate of Birjand in South Khorasan Province, combined with cultural factors (e.g., the consumption of raw vegetables and close contact with dogs), likely has a role in the local endemicity of CE. The semi-arid conditions and direct sunlight may reduce the environmental persistence

of *Echinococcus* eggs (11). Nevertheless, common established cultural and economic factors (e.g., extensive pastoral activities, close human-dog contact, and the dietary habit of consuming raw, potentially contaminated vegetables) create persistent transmission pathways that may counteract the limiting effects of the climate. While specific data for Birjand are limited, preliminary findings indicate that targeted research and control initiatives are necessary to address knowledge gaps and mitigate further transmission. It is noteworthy that a combination of radiological imaging and immunological assays is required for the diagnosis of CE. Ultrasonography, computed tomography, and magnetic resonance imaging remain primary imaging modalities for cyst detection and characterization. In addition, serologic techniques, such as enzyme-linked immunosorbent assay (ELISA) and immunoblot tests, provide supportive evidence by detecting circulating antibodies. Although imaging typically offers insight into lesion size, number, and location, serological tests are useful for screening at-risk populations, especially when there are limited imaging facilities. Nonetheless, false negative or positive results may arise, reminding researchers and clinicians of the ongoing need to refine and validate diagnostic protocols (12,13). Despite the long-standing recognition of the problem, CE persists as a critical public health concern in many regions, including parts of Iran where dogs and livestock share close quarters with humans (14). It should be noted that many infections remain undiagnosed due to the chronic nature of CE. These cases can progress to advanced stages, requiring more expensive and invasive treatments (1). Consequently, timely case-finding, accurate seroepidemiological assessment, and streamlined diagnostic methods are crucial to effective control. Therefore, the present study focuses on determining the seroepidemiological status of human hydatid cysts in Birjand and examining pertinent risk factors, such as contact with dogs, occupational exposures, dietary habits, and personal hygiene practices.

Materials and Methods

Study Design and Setting

This epidemiological, descriptive-analytical, cross-sectional study was conducted to estimate the seroepidemiological status of human hydatid cyst infection in Birjand, located in South Khorasan Province, Iran (Figure 1). The region is considered endemic for echinococcosis because of its semiarid climate and considerable pastoral activities involving sheep, goats, and other livestock (5,14). The study protocol was approved by the Research Ethics Committee of Birjand University of Medical Sciences (IR.BUMS.REC.1403), consistent with established requirements for securing ethical approval from an appropriate board when human participants are involved.

Study Population

A total of 400 individuals aged 10 years and older who presented for routine checkups at comprehensive health service centers in Birjand were recruited using systematic sampling from 2022 to early 2023. All participants provided written informed consent and demonstrated a willingness to participate in the study. To ensure data accuracy and minimize confounding factors, individuals were excluded if they had recently used anti-parasitic medications, which could alter serological results. Moreover, the other exclusion criteria included known underlying systemic diseases (e.g., severe hematologic conditions) that might interfere with immunological assays (1), failure to provide informed consent or complete the diagnostic tests (2), or provision of the serum samples of inadequate quality (e.g., gross haemolysis or hyperlipidaemia) that would

Figure 1. The Geographical Map of South Khorasan Province, Iran

compromise the validity of the ELISA test (3).

Sample Size Determination

The sample size was determined based on estimating an anticipated seroprevalence of 20% for echinococcosis (1). Considering a 95% confidence interval (1-α = 0.95) and a margin of error (d) of 0.04, the initial sample size was calculated to be 375. In addition, 400 participants were ultimately included to account for possible attrition or missing data.

Data Collection and Questionnaire

Upon obtaining consent, all participants were interviewed by trained research staff using a structured questionnaire. This questionnaire collected detailed demographic data (e.g., age, gender, education level, occupation, and rural vs. urban residence) and potential risk factors for CE (e.g., contact with dogs, consumption of raw vegetables, frequency of handwashing, and any relevant clinical history). The content validity of the questionnaire was confirmed by experts in parasitology and epidemiology. Further, pilot testing was conducted among 20 participants to ensure clarity and reliability.

Blood Sample Collection and Serum Preparation

Overall, 5 mL of venous blood was collected from each participant under strict aseptic conditions. Of these, approximately 2 mL were used for a routine complete blood count evaluation, and 3 mL were allowed to clot in a plain tube for serological testing. Then, the clotted tubes were centrifuged at 3,000 rpm for 10 minutes (14). Eventually, the separated serum samples were transferred into sterile, labelled microtubes and stored at −20 ˚C until further analysis.

Serologic Testing by Enzyme-Linked Immunosorbent Assay

All sera were tested for immunoglobulin G (IgG) antibodies against *E. granulosus* using a commercial ELISA kit (Pishtaz Teb, Tehran, Iran; Figure 2), which employs antigen B in a sandwich-ELISA format and has a reported sensitivity and specificity higher than 95% (12,13). According to the manufacturer's protocol, each serum sample was thawed at 25 ± 2˚C for 30–45 minutes, adhering to recognized practices that help preserve sample quality. The samples were then diluted and added to antigen B-coated wells, along with positive and negative controls, to detect anti-*Echinococcus* IgG. Furthermore, horseradish peroxidase-conjugated anti-human IgG and a 3,3',5,5'-tetramethylbenzidine substrate were subsequently employed, with multiple washing steps removing unbound components. Finally, optical density measurements at 450 nm enabled the classification of samples above the cutoff as positive and those below as negative. Based on established methods for determining test cutoffs in continuous measures, the threshold was set by subtracting the mean of the negative control and a designated factor, or per manufacturer guidance. The samples with results above and below the cutoff value were considered positive and negative, respectively. Any borderline samples were retested to ensure a definitive classification.

Statistical Analysis

The obtained data were analyzed using IBM SPSS Statistics software, version 22. Descriptive statistics (frequencies, percentages, means, and standard deviations) were utilized to summarize demographic and serological data. The associations between seropositivity for hydatid cysts and relevant independent variables (e.g., age, gender, education level, contact with dogs, handwashing habits,

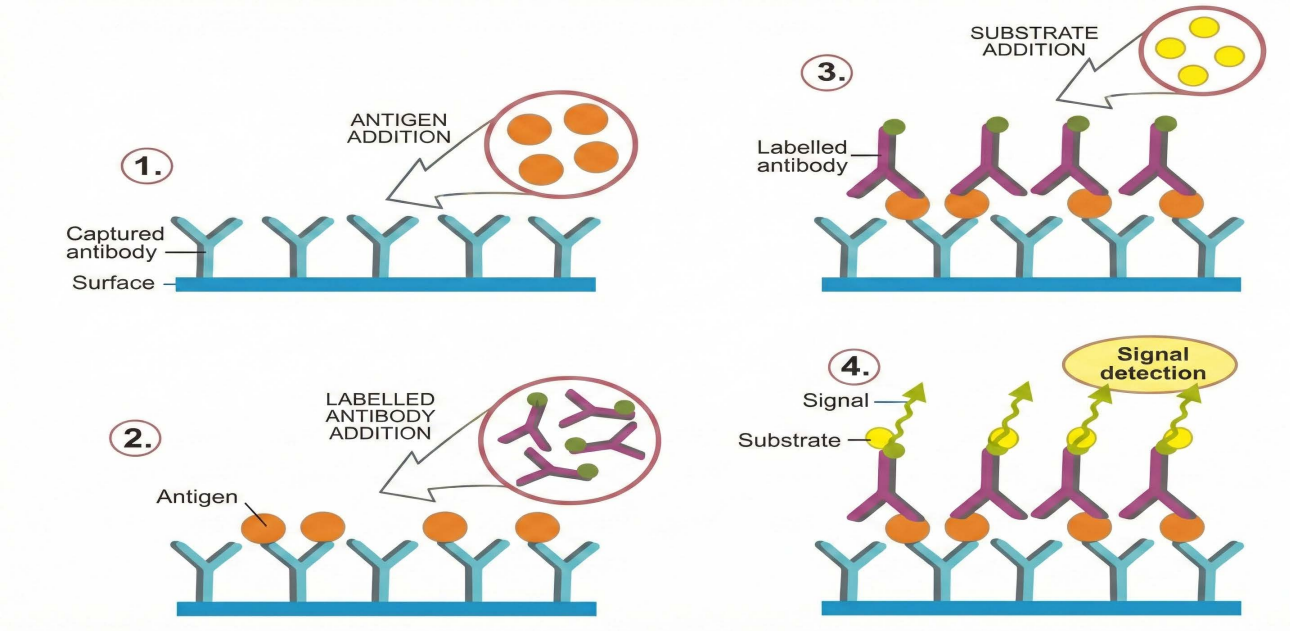

Figure 2. A Schematic Diagram of the Pishtaz Teb ELISA Kit Protocol. *Note*. ELISA: Enzyme-linked immunosorbent assay

and raw vegetable consumption) were assessed via chi-square (χ^2) tests or Fisher's exact tests, where appropriate. Additionally, logistic regression was employed to estimate odds ratios and 95% confidence intervals for multiple risk factors. A P value < 0.05 was considered statistically significant in all analyses.

Results

In general, 400 individuals who presented for periodic medical checkups at comprehensive health service centers in Birjand were enrolled in this cross-sectional investigation. The largest proportion of participants fell into the age group of 31–40 years old (25.5%), with a mean (SD) age of 39 years (±15). Of the total sample, 271 (67.8%) were female. Concerning education, 72.2% did not hold any university-level qualifications. Based on occupational status, the majority of the participants (73.7%) were neither farmers nor office workers but rather engaged in a broad category of "other" jobs (e.g., service sector, household work, and trade). Regarding the place of residence, 71.2% lived in urban areas, whereas 28.8% resided in rural settings. An additional subset of questions focused on behaviors potentially related to infection risk. Nearly 78% reported that they did not consistently wash their hands before meals, 72% denied contact with dogs, and 75.2% indicated that they generally did not consume raw vegetables (Table 1). Among the 400 examined serum samples, five tested positive for IgG antibodies against *E. granulosus* by ELISA, corresponding to an overall seroprevalence of 1.25% (5/400). No samples were detected as equivocal upon repeat testing (Table 1). Of the five seropositive individuals, three were within the 21–30-year age group (3.3% positivity in that subgroup), one person was over 50 years of age, and another fell into the 31–40-year age range. Comparative analyses revealed that the prevalence of echinococcosis did not significantly differ across predetermined age strata ($P > 0.05$). Based on the results, 3 out of 5 positive individuals were male (2.3% positivity among men compared to 0.7% positivity among women). Although the proportion was numerically higher in men, the difference was not statistically significant ($P > 0.05$). Of the five seropositive individuals, four did not have university-level education (1.3% positivity in this subpopulation), whereas one individual (0.9% positivity) mentioned holding a university degree. Thus, statistical comparison indicated no significant difference according to the education level ($P > 0.05$). Interestingly, three out of the five positive cases were in the "farmer" subgroup (8.5% prevalence among farmers) compared to 2/295 (less than 1%) in other occupational categories. Logistic regression uncovered a significant relationship between farming and seropositivity to hydatid cysts ($P < 0.05$). Three seropositive individuals (2.4%) lived in rural areas, while two (0.7%) were urban residents; the difference in positivity did not, however, reach statistical significance ($P > 0.05$). As regards handwashing, of the 400 participants, 78% reported that they did not regularly wash their hands before meals. Among the participants of this group, four individuals tested seropositive for *E. granulosus* (4/312, 1.3%). In contrast, only one positive case was noted among those who mentioned consistent handwashing (1/88, 1.1%). The association between irregular handwashing and echinococcosis reached statistical significance ($P < 0.05$) when adjusting for other

Table 1. Demographic Characteristics and *Echinococcus* IgG Seropositivity of Participants (N = 400)

Characteristic	Category	n (% of Total)	Positives n (%)	*P*-Value
Age group	<20	60 (15)	0 (0)	>0.05
	21–30	100 (25)	3 (3.0)	
	31–40	102 (25.5)	1 (1.0)	
	>40	138 (34.5)	1 (0.7)	
Gender	Male	129 (32.4)	3 (2.3)	>0.05
	Female	271 (67.6)	2 (0.7)	
Education	No university degree	289 (72.2)	4 (1.4)	>0.05
	University degree	111 (27.8)	1 (0.9)	
Occupation	Farmer	35 (8.7)	3 (8.5)	<0.05*
	Others (e.g., office worker)	365 (91.3)	2 (0.5)	
Place of residence	Urban	285 (71.2)	2 (0.7)	>0.05
	Rural	115 (28.8)	3 (2.6)	
Handwashing before meals	Inconsistent	312 (78)	4 (1.3)	<0.05*
	Consistent	88 (22)	0 (0)	
Consumption of raw vegetables	Yes	99 (24.8)	4 (4.0)	<0.05*
	No	301 (75.2)	1 (0.3)	
Contact with dogs	Yes	112 (28)	3 (2.7)	>0.05
	No	288 (72)	2 (0.7)	

Note. IgG: Immunoglobulin G. *Statistically significant at $P < 0.05$.

covariates in the model (Table 1). Regarding dog contact, while 72% of respondents indicated no close contact with dogs, three seropositive individuals (2.6%) had a history of dog ownership or frequent exposure to dogs, and two (0.7%) reported no canine contact. The difference failed to demonstrate statistical significance ($P>0.05$). In addition, among participants who mentioned regularly eating raw vegetables (n=160), four tested positive (2.5%). Contrarily, only one tested positive (0.4%) among those who rarely or never ate raw vegetables (n=240). The difference was statistically significant ($P<0.05$). Table 1 presents the demographic characteristics and *Echinococcus* IgG seropositivity of participants.

Discussion

CE is a globally significant zoonotic disease caused by the larval stages of *E. granulosus*, and imposes serious health and economic burdens on endemic regions (4,15). In this study, the overall seroprevalence of human hydatid cysts in the sampled population of Birjand was found to be relatively low (1.25%). Notwithstanding the modest seropositivity, these results underscore the continued need for surveillance, education, and preventive measures, given the chronic and often clinically silent progression of CE (9). A seropositivity prevalence of about 2% aligns well with the results of numerous earlier studies performed in Iran, although research has indicated a wide spectrum of seroprevalence figures—from under 1% to over 15% in certain areas (7). For example, serological studies conducted in Golestan, Ilam, and the southwestern areas of the country have revealed rates that are both comparable to and exceeding those in the current research (16,17). In comparison, the rate in Birjand was lower than that reported for the neighboring Kerman province (a prevalence of 7–8%) and was similar to the lower-end estimates from other adjacent provinces, such as Sistan and Baluchestan and Isfahan (both ≤5%) (18). This variability is mirrored in studies from other nations with comparable semi-arid climates and pastoral economies. For instance, the findings of a study in the rural Denizli province of Turkey demonstrated a CE seroprevalence of 6.9% among animal breeders, a group with high occupational exposure similar to the farmers in our study (19). Similarly, research in the Al-Mafraq governorate of Jordan, another region where livestock rearing is common, reported a seroprevalence of 4.1%, with a significant association found with the practice of slaughtering sheep inside homes (20). Differences in climate, culture (e.g., dog ownership and farming methods), public awareness, diagnostic methods, and sample sizes can explain the variation in seroprevalence. More importantly, it likely reflects the unique local interplay of climate and culture; for example, the semi-arid conditions in Birjand may limit egg survival compared to more temperate zones, while specific cultural habits (e.g., raw vegetable consumption and farming practices) sustain transmission and result in the observed prevalence rate (18). The slight variations in prevalence between rural (2.4%) and urban (2.1%) inhabitants in this study were not statistically significant. This lack of a distinct urban-rural separation has been observed in some other studies (21,22). Rural areas may frequently experience greater exposure to definitive hosts (dogs) and inadequate meat inspection practices; however, as Birjand progresses and lifestyles begin to align (e.g., the widespread movement of dogs and the sale of produce in both urban and rural markets), risk factors can become interchangeable between environments. Interactions with dogs and the intake of possibly contaminated vegetables underwent investigation. Although the findings of this study did not reveal any statistically significant link to dog ownership, earlier studies across Iran have often identified contact with dogs and the inappropriate disposal of infected organs as key risk factors for the transmission of *E. granulosus* (5,6). In contrast, inadequate washing of vegetables demonstrated a notable relationship with positive serology ($P<0.05$), which conforms to the findings of another research, emphasizing contaminated produce as a key pathway for the unintentional intake of infectious eggs (8). The highest seropositivity (3.3%) was noted in the 21–30-year age group, although this was not statistically significant. Multiple research findings suggest that the clinical signs of hydatid disease may present more frequently in adults, which may be partially due to the slow growth and extended incubation period of the parasite (2,23). Concerning gender, although some studies have reported elevated rates in females (potentially linked to greater engagement in home-related tasks such as gardening and cleaning produce), the current research indicated a marginally higher rate in males (2.3% compared to 0.7%), but this difference was not significant ($P>0.05$). This highlights that variations in exposure are likely influenced by local cultural and behavioral contexts, rather than by gender alone. While our findings have confirmed a low seroprevalence, this may represent a hidden burden of asymptomatic or subclinical infections, which can lead to significant long-term morbidity. Therefore, targeted interventions particularly focusing on rural practice, dog deworming and vaccination, improved slaughterhouse inspection, and public education, remain a cornerstone of hydatid disease control (3,18). Precise household-level interventions (e.g., ensuring proper washing of vegetables) could be pivotal in decreasing the burden.

Study Limitations

Several limitations inherent to this study should be acknowledged. The study relied on ELISA-based serodiagnosis, which has well-documented constraints while being sensitive and suitable for large-scale screening. For instance, IgG antibodies can persist for years, making it difficult to differentiate between a past, resolved infection and an active one. Furthermore, false-positive results may occur due to cross-reactivity with other helminth infections (e.g., *Taenia saginata*), while false-negative

results are possible in patients with early-stage infections or deeply encapsulated, calcified cysts that do not elicit a strong humoral immune response (9). Therefore, for clinical confirmation, these serological findings would require supplementation with imaging techniques (e.g., ultrasonography) or more specific confirmatory methods (e.g., immunoblotting). Moreover, future research can thoroughly address socioecological factors, such as stool examinations of definitive hosts, direct household observation of hygiene routines, and molecular analysis of *Echinococcus* strains circulating in the region (11,24,25).

Conclusion

The low but non-negligible seroprevalence of human hydatid cysts in Birjand highlights the endemic status of the disease and reaffirms the necessity of comprehensive strategies, including health education (e.g., safe disposal of offal, regular deworming of dogs, and proper washing of produce) to limit transmission. While the current burden seems less severe than in certain other parts of Iran, the potential for hidden or subclinical infections cautions regional health authorities to remain vigilant and proactive. Furthermore, it is crucial to adopt a "One Health" approach by enhancing collaboration with veterinary services to implement systematic dog deworming and manage stray dog populations while also enforcing stricter regulations at local abattoirs for the safe inspection and disposal of potentially infected livestock offal to interrupt the parasite's lifecycle.

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Authors' Contribution

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Formal analysis: M Mousavi.
Investigation: Z Naderi, H Kabiri.
Methodology: Z Naderi, H Kabiri.
Project administration: A Tavakoli Kareshk.
Resources: A Tavakoli Kareshk.
Supervision: A Tavakoli Kareshk, M Mousavi.
Software: M Mousavi.
Validation: Z Naderi, H Kabiri.
Writing–original draft: Z Naderi, H Kabiri, R Solgi.
Writing–review & editing: A Tavakoli Kareshk, M Mousavi.

Competing Interests

The authors have no competing interests to declare that are relevant to the content of this article.

Ethical Approval

The study protocol was approved by the Ethics Committee of Birjand University of Medical Sciences Research (IR.BUMS.REC.1403), in line with established requirements for securing ethical approval from an authorized board when human participants are involved.

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Informed Consent

Not applicable.

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