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# Investigation of Seroprevalence of Hydatidosis in High-risk Individuals in Sistan and Baluchestan Province, Southeast of Iran

**Davood Anvari[1,2,3], Seyed Abdollah Hosseini[2], Ahmad Daryani[2], Shahabbedin Sarvi[2], Adel Spotin[4], Sanaz Vaziri Shahraki[5], Mohammad Kalkali[3], Abolghasem Siyadatpanah[6], Shirzad Gholami[2*]**

*1. Student Research Committee, Mazandaran University of Medical Science, Sari, Iran.*
*2. Department of Parasitology, Toxoplasmosis Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.*
*3. Department of Parasitology, School of Medicine, Iranshahr University of Medical Sciences, Iranshahr, Iran.*
*4. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*
*5. Department of Parasitology Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran.*
*6. Department of Nursing, Ferdows School of Health and Allied Medical Sciences, Birjand University of Medical Sciences, Birjand, Iran.*

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## ABSTRACT

**Background:** Hydatidosis is known as one of the most prevalent zoonotic diseases across the world. This complication is also endemic in Iran, followed by a higher risk of infection in rural areas. To our knowledge, there has been no study on the seroprevalence of hydatidosis in Sistan and Baluchistan Province, Southeast of Iran. The main objective of the current study was to examine the seroprevalence of hydatidosis and its risk factors in high-risk individuals (farmers and ranchers) living in Sistan and Baluchistan Province.

**Materials and Methods:** This study included 500 serum samples, and the participants were requested to complete a researcher-made questionnaire. Subsequently, counter-current immunoelectrophoresis (CCIEP) and enzyme-linked immunosorbent assay (ELISA) methods were employed to analyze the anti-*Echinococcus granulosus* antibody. The analysis of the obtained data was conducted by logistic regression in SPSS software, version 22.

**Results:** According to the results, four (0.8%) cases were found positive for anti-*E. granulosus* antibody by both CCIEP and ELISA tests. Seroprevalence of hydatidosis was more in rural people, compared to those in urban areas. It was also higher in illiterate people than in educated people. Nevertheless, seropositivity showed no significant differences with age, gender, occupational status, education level, place of residence, and contact with dogs (P>0.05).

**Conclusion:** The prevalence rate of hydatidosis in Sistan and Baluchistan Province was similar to that in neighboring provinces. According to the findings, high-risk individuals offer remarkable information about the epidemiology of hydatidosis in Sistan and Baluchistan province in southeastern Iran. This could help to manage and prevent this infection.

*** Corresponding Author:**
*Shirzad Gholami, Professor.*
***Address:*** *Department of Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.*
***Phone:*** *+98 (151) 3543087*
***E-mail:*** *sgholami200@gmail.com*

## Introduction

Cystic echinococcosis (CE) or hydatidosis belongs to the genus *Echinococcus* owing to the larval form of several species. It is also among the main zoonotic diseases across the world leading to problems for public health and remarkable economic losses. According to the World Health Organization (WHO), echinococcosis is among the neglected tropical diseases [1]. *Echinococcus granulosus* is the most common species of *Echinococcus* infecting humans globally. It is a complex of species and genotypes with different life cycle patterns and host ranges [2-6]. Humans are infected by the ingestion of helminths' eggs in such various manners as consuming contaminated vegetables with canid hosts' stool or wild/domestic livestock's viscera containing hydatid cysts [7]. This disease is prevalent in various sheep farming regions, such as Asia, Eastern and Southern Europe, New Zealand, Australia, Mediterranean coasts, South America, and the Middle East (including Iran) [7-9].

According to WHO, echinococcosis is native to Iran, and several studies have approved this claim [10, 11]. Moreover, it was estimated that 635232 asymptomatic cases live in Iran, and the overall cystic echinococcosis cost is estimated at US$ 232.3 million annually [12, 13]. Several research projects have been carried out in various parts of Iran to determine the human hydatidosis seroprevalence [10, 14]. The prevalence rate of this disease has been estimated to be within the range of 1.2%-31.6% in different provinces of Iran [10, 15, 16]. The disease might be diagnosed even 20-25 years after the infection since the asymptomatic period is too long [7, 17].

Ultrasound imaging, clinical symptoms, and immunological methods are among the techniques to diagnose human hydatidosis. Counter-current immunoelectrophoresis (CCIEP) is widely used as a rapid, simple, and affordable immunoassay for the detection of antibodies as well as antigens in a variety of parasitic infections such as echinococcosis [18-21]. The specificity and the sensitivity of the CCIEP method in diagnosing hydatid cyst infection are estimated at 68.9%-100% and 86.7%, respectively [22-24]. To the best of the authors' knowledge and regarding the remarkable economic and medical significance of the disease, there is a dearth of research on the epidemiological status of this complication in all entire regions of Sistan and Baluchistan province. Therefore, the present research aimed to ascertain the prevalence of hydatidosis in high-risk individuals in Sistan and Baluchistan province by the CCIEP method and also, due to the higher specificity and sensitivity of enzyme-linked immune sorbent assay (ELISA) in the diagnosis of this disease [25, 26], in the present study, the ELISA test was used to confirm the samples tested with CCIEP test definitively.

## Materials and Methods

### Samples

The current descriptive cross-sectional research was carried out from March to July 2021 in Sistan and Baluchistan Province, Southeast Iran (Figure 1).

Five hundred Samples were collected from randomly high-risk individuals (Farmers and Ranchers) from five cities (Zabol, Zahedan, Iranshahr, Saravan, and Chabahar). Written informed consent forms were obtained from subjects before the start of the research. Questionnaires were utilized to collect demographic characteristics and relevant history data. The collected data covered such information as age, gender, place of residency (rural/urban), occupation, literacy, and history of contact with dogs. For the purposes of the study, 3 mL sample of venous blood was obtained from each case. Following that, at 3000 rpm centrifugation for 5 min, the sera were separated and stored at -20°C. At low temperatures, for additional analysis, the sera were transferred to a parasitology laboratory affiliated to Mazandaran University of Medical Sciences, Sari, Iran.

### Antigen

Hydatid cysts that were obtained from infected livers of sheep were utilized to aspirate crude hydatid cyst fluid (HCF Ag). Following that, Antigen B was purified and extracted, and to eliminate particles, at 1500 g, 100 mL of HCF was centrifuged for 30 min, and against 0.005 M acetate buffer (Merck, Germany) (pH=5), the supernatant was dialyzed overnight at 4°C. It is worth mentioning that this step was repeated once. An ultracentrifuge was used at 30,000 g to centrifuge the content of dialysis bags at 4°C for 30 minutes. This procedure settles insoluble proteins (e.g., Ag5 and AgB). In the next stage, in order to eliminate globulins, 10 mL of 0.2 M phosphate buffer (pH=8) (Merck, Germany) was used to dissolve the precipitates. Then, 2.31 g of ammonium sulfate powder (Merck, Germany) was gradually added, and the mixture was shaken to saturate the preparation with ammonium sulfate (40%). After a short interval, at 3000 g, the preparation was then centrifuged for 30 min. Next, for 15 min, the supernatant was incubated in a water bath. At this stage, due to its sensitivity to heat, Ag5

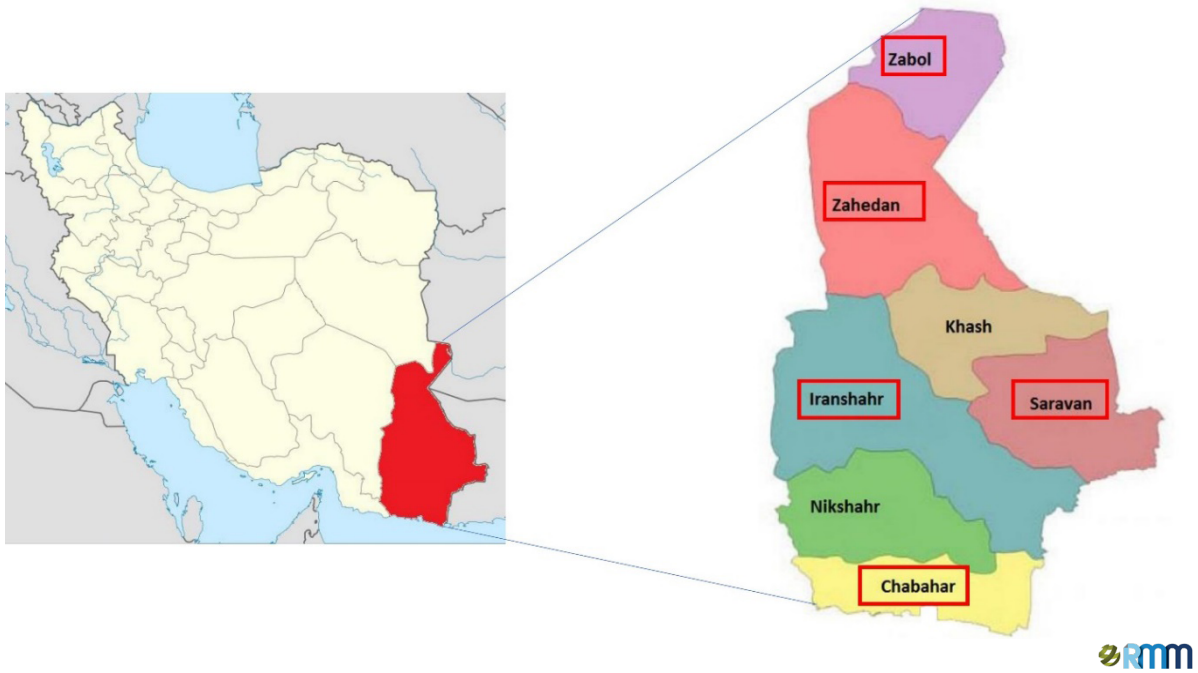


**Figure 1.** Geographical location of Sistan and Baluchestan province

became denatured and insoluble. For 1 hour, the mixture was then centrifuged by an ultracentrifuge at 30,000 g, and a 0.2 µm filter was utilized to collect and filter the supernatant containing AgB. Afterward, sodium azide (NaN3) (Ojazma, Iran) was added, and the mixture was stored at -70°C until further utilization [27].

## Counter-current immunoelectrophoresis test (CCIEP)

The test was conducted following the method previously described considering some modifications [19], and in tris borate buffer with pH 8.2 (Merck, Germany), the Agarose was prepared, a half gram of which (Pishgam, Iran) was added to 25 mL of tris borate buffer, and it was then heated.

Then, molten buffer gel (5 mL) was poured onto glass slides, and it was subsequently let to solidify. Furthermore, two four mm-diameter wells were cut with a well distance of six mm for trials with corresponding positive serum collected from patients with hydatid cysts who underwent surgery (positive sample). To screen serum samples, two rows were used that contained four wells. Each well had a 4 mm cut with a distance of 6 mm by another well and sealed bases with molten agar. Antigen B of E. granulosus was used to fill the well that was supposed to be located on the cathodic slide, and the other well (anodic well) was filled with the corresponding serum of high-risk individuals. 10 mL of antigen B and serum of high-risk individuals were used in this test as well as positive (serum collected from patients with hydatid cysts who underwent surgery) and negative (Distilled water) control samples. Electrophoresis was conducted in an appropriate trough that contained barbitone buffer. A rack was utilized to place the slides on, and each end was connected with a filter paper wick that was dipped in a buffer. For 90 min, a 50-mA current was applied per slide. Before and after staining, the observations were recorded, and normal saline was employed to wash the slides, and they were subsequently stained with Coomassie brilliant blue stain (Merck, Germany).

## ELISA test

The ELISA test was used to confirm the samples tested with the CCIEP test definitively. Also, 250 negative samples in the CCIEP test were randomly analyzed by ELISA. Using an ELISA kit (PishtazTeb, Iran), the samples were examined in terms of the *Echinococcus* IgG in 96-well microplates. The IgG-ELISA test was also conducted following the manufacturer's instructions, and two operators read each test independently. An ELISA plate reader (State Fax® 2100, Awareness, USA) was used to measure the optical density (OD) at 492 nm. In total, 30 sera collected from healthy volunteers in previous research were examined to measure the cut-off point that was assigned as 3SD above the mean value of controls [28].

## Statistical analysis

Through logistic regression, the odds ratio (OR) and 95% confidence interval (CI) were estimated to investi-

gate the association of the risk of human cystic echinococcosis with each variable. The obtained data were analyzed in SPSS software version 22, Chicago, IL, USA, and a P<0.05 was considered statistically significant.

## Results

In the current study, 500 serum samples from farmers and ranchers of Sistan and Baluchestan province were examined by CCIEP test. The results demonstrated that 4 serum samples were identified as positive (0.8%) (Figure 2). Moreover, all these four samples were also confirmed by the ELISA method, and all random samples were negative. Also, among all regions, the highest prevalence of infection was observed in Iranshahr city (Table1).

In the current study, out of 500 serum samples, 315 (63%) were male and 185 were female (37%). In addition, 67 cases lived in urban areas (13.4%) and 433 cases lived in rural areas (86.6%). The prevalence of infection was higher in men than women, and people living in rural areas than urban areas, but this difference was not significant. In addition, the prevalence of infection was higher in people under 60 years old than in people over 60 years old and people without education than in educated people. However, none of these cases was statistically significant. Out of 500 serum samples, 209 were farmers (41.8%), and 291 were ranchers (58.2%). Based on the results of the present study, hydatidosis infection was more prevalent in ranchers than farmers, and those who were exposed to dogs showed a higher prevalence of infection than those who had no contact with dogs. The difference between hydatid infection

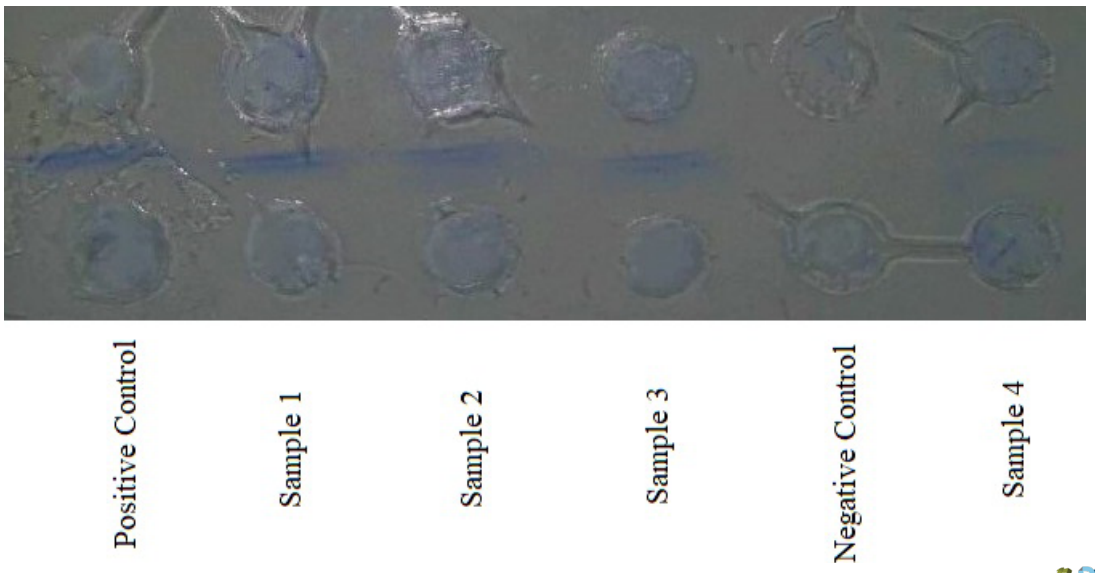


**Figure 2.** CCIEP method on 1% agarose gel slide for detection of anti-*E. granulosus* antibodies in the serum of high-risk individuals living in Sistan and Baluchestan Province

**Table 1.** Prevalence of hydatidosis infection in different regions of Sistan and Baluchestan province using CCIEP and ELISA methods

| Locations | No. | | Prevalence |
|---|---|---|---|
| | Examined | Positive | |
| Zabol | 100 | 1 | 0.2 |
| Zahedan | 100 | 1 | 0.2 |
| Iranshahr | 100 | 2 | 0.4 |
| Saravan | 100 | 0 | 0.0 |
| Chabahar | 100 | 0 | 0.0 |
| Total | 500 | 4 | 0.8 |

and all of mentioned variables were not statistically significant (P>0.05) (Table 2).

## Discussion

The CE is among the major parasitic diseases of zoonoses in Iran and has become endemic in most parts of this country. However, there is little information about the epidemiology of this disease, especially in high-risk individuals in Iran. Serological tests are widely used because of their low cost, speed of response, and easy of performing on a large number of serum samples. The present study, among the high-risk population in Sistan and Baluchestan province, estimated the serum prevalence of CE-infected individuals (farmers and ranchers) at 0.8%.

Previous research in various parts of Iran shows the prevalence rate of human CE infection according to serological methods, particularly ELISA, in the general population from 1.2% to 21.4% in different regions of Iran [14]. In 2016, Shafiei et al., in a systematic review and meta-analysis study, estimated and reported a serum prevalence of human CE infection in Iran of 6% [10]. Nevertheless, in 2018, Khalkhali et al., in another systematic review and meta-analysis, estimated the prevalence of human CE infection in Iran at 4.2%. They also estimated the prevalence of CE in the northern regions at 4.4%, the southern regions at 5.8%, the central regions at 2.2%, and the western regions at 5%. However, did not find any studies related to hydatidosis infection in the eastern region of the country such as Sistan and Baluchestan province [29]. Moreover, in Kerman province, which is adjacent to Sistan and Baluchestan province, the prevalence of CE infection in people referring to medical centers was estimated at 1.85%. In current research, the prevalence of infection in Sistan and Baluchestan province was estimated at 0.8%, which is similar to infection in neighboring areas [30].

The present study showed that men were more at risk of infection than women and had a higher prevalence of CE. The reason might be local cultures and male behavioral tasks, including farming and herding as well as keeping and feeding dogs, and men are more exposed to environmental risk factors, compared to women. Although many studies in Iran and other countries showed

**Table 2.** Logistic regression analysis of positive serology subjects

| Variables | | No. (%) | | Odds Ratio (95% CI*) | P |
|---|---|---|---|---|---|
| | | Examined | Positive | | |
| Sex | Male | 315(63.00) | 3(0.6) | Reference** | >0.9999 |
| | Female | 185(37.00) | 1(0.2) | 1.76(0.14-93.37) | |
| Age (y) | 30-60 | 373(74.60) | 3(0.6) | Reference | >0.9999 |
| | ≥60 | 127(25.40) | 1(0.2) | 1.02(0.08-54.05) | |
| Residence | Urban | 67(13.40) | 1(0.2) | Reference | 0.4386 |
| | Rural | 433(86.60) | 1(0.2) | 2.16(0.04-27.45) | |
| Education | Educated*** | 143(28.60) | 1(0.2) | Reference | >0.9999 |
| | Non-educated | 357(71.40) | 3(0.6) | 0.83(0.01-10.45) | |
| Occupation | Farmer | 209(41.80) | 1(0.2) | Reference | 0.6436 |
| | Rancher | 291(58.20) | 3(0.6) | 0.46(0.00-5.80) | |
| Contact with dog | Yes | 362(72.40) | 3(0.6) | Reference | >0.9999 |
| | No | 138(27.60) | 1(0.2) | 1.14(0.09-60.53) | |
| Total | | 500(100) | 4(0.8) | - | - |

* CI: Confidence interval; ** Reference: the level of variable, which other levels compared with it; *** Educated: defines as illiterate, primary, secondary school, college and above.

that the prevalence of infection in women is higher than in men [31-34], but Shahrokhabadi et al. in Kerman province and Zibaei et al. in western Iran indicated that hydatid infection was be significantly higher in men than women [30, 35]. In general, this difference must be investigated in further cultural and environmental contexts in future studies.

The results of current research demonstrated that the prevalence of infection in the age range of 30 to 60 years is higher than in the age group over 60 years. Other studies in Iran and other parts of the world have shown that CE most commonly occurs in middle life [36-39]. It has also been shown that the lack of serum positivity in infections might be due to low activity and low immune response in older people [5, 40-42]. In the present study, this may be due to the behavioral and work pattern and more contact of farmers and ranchers with risk factors for this infection, so that at this age, more time due to more physical strength and heavier tasks to spend on work related to agriculture and livestock and are more at risk of contracting this infection.

In the present study the prevalence of CE infection was more prevalent in rural area residents, compared to urban area residents. So that people living in rural areas were 2.16 times more at risk than people living in urban areas (95% CI=0.04-27.45) (OR=2.16). The findings of this research were similar to previous studies in other regions of Iran as well as other countries [14, 32, 43]. This may be due to lifestyle patterns and exposure to risk factors such as infected dogs, water and food, contaminated vegetables, and other factors [14, 17]. Nevertheless, it is evident that in urban areas, personal, social, and environmental health is significantly higher than in rural areas, and the risk factors for this infection (such as contact with infected dogs, water, food, and contaminated vegetables, etc.) are less than in rural areas.

In this research, the highest rate of CE infection was found in illiterate people. In some studies, the prevalence of CE infection in educated people is higher than in illiterate people [15, 44]. However, in the study by Baharsefat et al. [45] and Fathi et al. [46], the prevalence of CE infection was more reported among illiterate people. A possible reason for this may be the lack of knowledge of the principles and health care among illiterate people. On the other hands, contact with predisposing factors (such as water, soil, infected dogs, etc.) occurs more, and there will be no sensitivity in this matter.

In this research, the prevalence of CE infection was higher among ranchers than farmers. In a study by Ziaei et al., 2017 in Mazandaran province, the prevalence of CE infection was higher among ranchers than farmers. However, no statistically significant difference could be found [15]. Also, in another studies the prevalence of hydatid disease was highest among ranchers [30]. A possible reason for this may be more concerning the infected herd dog. Because usually all ranchers keep dogs to take care of their livestock, and all of these dogs had not received antiparasitic drugs. Also, in the feeding of these dogs, sometimes the viscera of slaughtered animals were used, which may be infected with hydatid cysts. In addition, these dogs were kept in the yard and were in close contact with their owners.

In this research, the highest rate of CE infection was found in people in contact with dogs. This situation is consistent with most of the research conducted in Iran and other countries [15, 46]. Universally, the dog is the animal that has adapted itself to human habitation more successfully than others [47, 48]. Despite today's lifestyle and close association with dogs and adherence to health principles and care, dogs may still be a major public health hazard. The findings of the current research showed that contact with dogs is possibly one of the major risk factors for hydatid disease.

## Conclusion

The results of the present study on high-risk individuals (farmers and ranchers) , offer remarkable information about the epidemiology of hydatidosis from Sistan and Baluchestan province in southeastern Iran. This could help to manage and prevent this infection. The CCIEP method is a simple method with high sensitivity and specificity that can well detect hydatidosis infection. Moreover, health education, hygienic and appropriate disposal of contaminated slaughterhouse waste, more attention to dog hygiene, usage of antiparasitic drugs and using hygienic water and vegetables as essential and vital solutions to control and reduce the rate of CE infection in humans and animals should be considered in this area. Also, further studies on the entire population at risk in the province and different age and occupational groups and attention to the risk factors involved are necessary.

## Ethical Considerations

### Compliance with ethical guidelines

Current study was approved by the Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran (Code: IR.MAZUMS..REC.1398.4712).

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### Authors contribution's

Conceptualization and Supervision: Shirzad Gholami, Ahmad Daryani, Shahabbedin Sarvi, Adel Spotin and Abolghasem Siyadatpanah; Methodology: Davood Anvari, Shirzad Gholami and Seyed Abdollah Hosseini; Investigation, Writing–original draft, and Writing–review & editing: All authors; Sample collection: Davood Anvari, Sanaz Vaziri Shahraki and Mohammad Kalkali; Data analysis: Adel Spotin and Seyed Abdollah Hosseini; Funding acquisition and Resources: Shirzad Gholami

### Conflict of interest

The authors declare no conflict of interest.

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## References

[1] Deplazes P, Rinaldi L, Alvarez Rojas CA, Torgerson PR, Harandi MF, Romig T, et al. Global distribution of alveolar and cystic echinococcosis. Adv Parasitol. 2017; 95:315-493. [DOI:10.1016/bs.apar.2016.11.001] [PMID]

[2] Thompson RCA. The taxonomy, phylogeny and transmission of echinococcus. Exp Parasitol. 2008; 119(4):439-46. [DOI:10.1016/j.exppara.2008.04.016] [PMID]

[3] Alizadeh Z, Mahami-Oskouei M, Spotin A, Kazemi T, Ahmadpour E, Cai P, et al. Parasite-derived microRNAs in plasma as novel promising biomarkers for the early detection of hydatid cyst infection and post-surgery follow-up. Acta Trop. 2020; 202:105255. [DOI:10.1016/j.actatropica.2019.105255] [PMID]

[4] Gessese AT. Review on epidemiology and public health significance of hydatidosis. Vet Med Int. 2020; 2020:8859116. [DOI:10.1155/2020/8859116] [PMID] [PMCID]

[5] Anvari D, Rezaei F, Ashouri A, Rezaei S, Majidiani H, Pagheh AS, et al. Current situation and future prospects of echinococcus granulosus vaccine candidates: A systematic review. Transbound Emerg Dis. 2021; 68(3):1080-96. [DOI:10.1111/tbed.13772] [PMID]

[6] Anvari D, Pourmalek N, Rezaei S, Fotovati A, Hosseini SA, Daryani A, et al. The global status and genetic characterization of hydatidosis in camels (*Camelus dromedarius*): A systematic literature review with meta-analysis based on published papers. Parasitology. 2021; 148(3):259-73. [DOI:10.1017/S0031182020001705] [PMID]

[7] Wen H, Vuitton L, Tuxun T, Li J, Vuitton DA, Zhang W, et al. Echinococcosis: Advances in the 21st century. Clin Microbiol Rev. 2019; 32(2):e00075-18. [DOI:10.1128/CMR.00075-18] [PMID] [PMCID]

[8] Pakala T, Molina M, Wu GY. Hepatic echinococcal cysts: A review. J Clin Transl Hepatol. 2016; 4(1):39-46. [DOI:10.14218/JCTH.2015.00036] [PMID] [PMCID]

[9] Ito A, Budke CM. The echinococcoses in Asia: The present situation. Acta Trop. 2017; 176:11-21. [DOI:10.1016/j.actatropica.2017.07.013] [PMID]

[10] Shafiei R, Hosseini Teshnizi S, Kalantar K, Gholami M, Mirzaee G, Mirzaee F. The seroprevalence of human cystic echinococcosis in Iran: A systematic review and meta-analysis study. J Parasitol Res. 2016; 2016:1425147. [DOI:10.1155/2016/1425147] [PMID] [PMCID]

[11] Khademvatan S, Majidiani H, Foroutan M, Hazrati Tappeh K, Aryamand S, Khalkhali HR. Echinococcus granulosus genotypes in Iran: A systematic review. J Helminthol. 2019; 93(2):131-38. [DOI:10.1017/S0022149X18000275] [PMID]

[12] Fasihi Harandi M, Budke CM, Rostami S. The monetary burden of cystic echinococcosis in Iran. Plos Negl Trop Dis. 2012; 6(11):e1915. [DOI:10.1371/journal.pntd.0001915] [PMID] [PMCID]

[13] Widdicombe J, Basáñez MG, Entezami M, Jackson D, Larrieu E, Prada JM. The economic evaluation of cystic echinococcosis control strategies focused on zoonotic hosts: A scoping review. Plos Negl Trop Dis. 2022; 16(7):e0010568. [DOI:10.1371/journal.pntd.0010568] [PMID] [PMCID]

[14] Mahmoudi S, Mamishi S, Banar M, Pourakbari B, Keshavarz H. Epidemiology of echinococcosis in Iran: A systematic review and meta-analysis. BMC Infect Dis. 2019; 19(1):929. [DOI:10.1186/s12879-019-4458-5] [PMID] [PMCID]

[15] Ziaei Hezarjaribi H, Fakhar M, Rahimi Esboei B, Soosaraei M, Ghorbani A, Nabyan N, et al. Serological evidence of human cystic echinococcosis and associated risk factors among general population in Mazandaran Province, northern Iran. Ann Med Surg. 2017; 18:1-5. [DOI:10.1016/j.amsu.2017.04.012] [PMID] [PMCID]

[16] Galeh TM, Spotin A, Mahami-Oskouei M, Carmena D, Rahimi MT, Barac A, et al. The seroprevalence rate and population genetic structure of human cystic echinococcosis in the Middle East: A systematic review and meta-analysis. Int J Surg. 2018; 51:39-48. [DOI:10.1016/j.ijsu.2018.01.025] [PMID]

[17] Garcia LS. Diagnostic medical parasitology. Hoboken: John Wiley & Sons; 2015. [DOI:10.1128/9781555819002]

[18] Shariff GM, Parija SC. Counter-current immunoelectrophoresis test for serodiagnosis of hydatid disease by detection of circulating hydatid antigen. J Microbiol Methods. 1991; 14(2):71-6. [DOI:10.1016/0167-7012(91)90035-O]

[19] Ananda KJ, D'Souza PE. Immunodiagnosis of echinococcus granulosus infection in dogs by counter immunoelectrophoresis (CIEP). J Parasit Dis. 2015; 39(4):654-7. [DOI:10.1007/s12639-014-0418-6] [PMID] [PMCID]

[20] Javaregowda AK, Naravi Y. Immuno-diagnosis of dirofilaria repens infection in dogs by counter immunoelectrophoresis (CIEP). J Vet Parasitol. 2019; 33(1):22-7. [DOI:10.5958/0974-0813.2019.00005.6]

[21] Elamin OHS, Ali MA, Elgezuli IAA, Bashir MA, Kahwa I. Diagnosis of pre-and post-treatment of echinococcus granulosus with counter current immune electrophoresis and bacterial co-agglutination. J Microbil Infect Dis. 2021; 11(2):88-94. [DOI:10.5799/jmid.951600]

[22] Ravinder PT, Parija SC. Countercurrent immunoelectrophoresis test for detection of hydatid antigen in the fluid from hydatid cysts: A preliminary report. Acta Trop. 1997; 66(3):169-73. [DOI:10.1016/S0001-706X(97)00036-3]

[23] Maleki F, Sarafpoor S. [Assessment of pure and B hydatid cyst fluid antigens for the diagnosis of hydatidosis (Persian)]. J Gorgan Univ Med Sci. 2014; 16(2):74-81. [Link]

[24] Siddartha PV, Babu AJ, Rao TM, Rayulu VC, Swetha CS. A comparative evaluation of four different immunoassays in the diagnosis of cystic echinococcosis using a crude and purified hydatid cyst fluid antigen. Acta Parasitol. 2022; 67(4):1667-79. [DOI:10.1007/s11686-022-00614-5] [PMID]

[25] Kalantari E, Bandehpour M, Pazoki R, Taghipoor-Lailabadi N, Khazan H, Mosaffa N, et al. Application of recombinant echinococcus granulosus antigen B to ELISA kits for diagnosing hydatidosis. Parasitol Res. 2010; 106(4):847-51. [DOI:10.1007/s00436-010-1726-0] [PMID]

[26] El-Kattan AM, M Abdel-Ra'ouf A, Yousef R, AbouElnga T, Mousa W, et al. Sensitivity and specificity of indirect enzyme linked immuno sorbent assay (ELISA) for diagnosis of hydatidosis in dromedary camels using hydatid cyst fluid antigens. J Vet Med Res. 2020; 27(1):66-75. [DOI:10.21608/jvmr.2020.69696]

[27] Shirazi S, Madani R, Hoghooghi Rad N, Ranjbar S. Isolation and purification of echinococcus granulosus antigen B from hydatid cyst fluid using three different methods. Arch Razi Inst. 2016; 71(2):103-8. [DOI:10.22034/ARI.2016.106448]

[28] Ilbeigi P, Mohebali M, Kia EB, Saber-Inasab M, Aryaeipour M, Bizhani N, at al. Seroepidemiology of human hydatidosis using AgB-ELISA test in Isfahan city and suburb areas, Isfahan Province, central Iran. Iran J Public Health. 2015; 44(9):1219-24. [PMID] [PMCID]

[29] Khalkhali HR, Foroutan M, Khademvatan S, Majidiani H, Aryamand S, Khezri P, et al. Prevalence of cystic echinococcosis in Iran: A systematic review and meta-analysis. J Helminthol. 2018; 92(3):260-8. [DOI:10.1017/S0022149X17000463] [PMID]

[30] Shahrokhabadi R, Rahimi E, Poursahebi R. Seroepidemiological study of human hydatidosis in Rafsanjan, Kerman. Zahedan J Res Med Sci. 2014; 16:46. [Link]

[31] Harandi MF, Moazezi SS, Saba M, Grimm F, Kamyabi H, Sheikhzadeh F, et al. Sonographical and serological survey of human cystic echinococcosis and analysis of risk factors associated with seroconversion in rural communities of Kerman, Iran. Zoonoses Public Health. 2011; 58(8):582-8. [DOI:10.1111/j.1863-2378.2011.01407.x] [PMID]

[32] Akalin S, Kutlu SS, Caylak SD, Onal O, Kaya S, Bozkurt AI. Seroprevalence of human cystic echinococcosis and risk factors in animal breeders in rural communities in Denizli, Turkey. J Infect Dev Ctries. 2014; 8(9):1188-94. [DOI:10.3855/jidc.4343] [PMID]

[33] Heidari Z, Mohammadi-Ghalehbin B, Alizadeh Z, Molaei S, Dogaheh HP, Mirzanejad-Asl H. Seroprevalence of Human hydatidosis in Ardabil Province, north-west of Iran. Iran J Parasitol. 2021; 16(4):593-600. [DOI:10.18502/ijpa.v16i4.7872] [PMID] [PMCID]

[34] Khan SN, Ali R, Khan S, Norin S, Rooman M, Akbar NU, et al. Cystic echinococcosis: An emerging zoonosis in southern regions of Khyber Pakhtunkhwa, Pakistan. BMC Vet Res. 2021;17(1):139. [DOI:10.1186/s12917-021-02830-z] [PMID] [PMCID]

[35] Zibaei M, Azargoon A, Ataie-Khorasgani M, Ghanadi K, Sadjjadi SM. The serological study of cystic echinococcosis and assessment of surgical cases during 5 years (2007-2011) in Khorram Abad, Iran. Niger J Clin Pract. 2013; 16(2):221-5. [DOI:10.4103/1119-3077.110156] [PMID]

[36] Rafiei A, Hemadi A, Maraghi S, Kaikhaei B, Craig PS. Human cystic echinococcosis in nomads of south-west Islamic Republic of Iran. East Mediterr Health J. 2007; 13(1):41-8. [PMID]

[37] Sarkari B, Sadjjadi SM, Beheshtian MM, Aghaee M, Sedaghat F. Human cystic echinococcosis in Yasuj district in southwest of Iran: An epidemiological study of seroprevalence and surgical cases over a ten-year period. Zoonoses Public Health. 2010; 57(2):146-50. [DOI:10.1111/j.1863-2378.2008.01200.x] [PMID]

[38] Muhammedoğlu B, Pircanoğlu EM, Pişkin E, Torun S, Karadağ M, Topuz S, et al. Treatment of hepatic hydatid disease: Role of surgery, ERCP, and percutaneous drainage: A retrospective study. Surg Laparosc Endosc Percutan Tech. 2021; 31(3):313-20. [DOI:10.1097/SLE.0000000000000861] [PMID]

[39] Gholami S, Tanzifi A, Sharif M, Daryani A, Rahimi MT, Mirshafiee S, et al. Demographic aspects of human hydatidosis in Iranian general population based on serology: A systematic review and meta-analysis. Vet World. 2018; 11(10):1385-96. [DOI:10.14202/vetworld.2018.1385-1396] [PMID] [PMCID]

[40] Tiaoying L, Jiamin Q, Wen Y, Craig PS, Xingwang C, Ning X, et al. Echinococcosis in Tibetan populations, western Sichuan Province, China. Emerg Infect Dis. 2005; 11(12):1866-73. [DOI:10.3201/eid1112.050079] [PMID] [PMCID]

[41] Weyh C, Krüger K, Strasser B. Physical activity and diet shape the immune system during aging. Nutrients. 2020; 12(3):622. [DOI:10.3390/nu12030622] [PMID] [PMCID]

[42] Crooke SN, Ovsyannikova IG, Poland GA, Kennedy RB. Immunosenescence and human vaccine immune responses. Immun Ageing. 2019; 16:25. [DOI:10.1186/s12979-019-0164-9] [PMID] [PMCID]

[43] Siyadatpanah A, Daryani A, Sarvi S, Spotin A, Sharif M, Esmaeelzadeh Dizaji R, et al. Phylogeography and genetic diversity of human hydatidosis in bordering the Caspian Sea, northern Iran by focusing on echinococcus granulosus sensu stricto complex. Iran J Public Health. 2020; 49(9):1758-68. [DOI:10.18502/ijph.v49i9.4097] [PMID] [PMCID]

[44] Bahram N, Sharbatkhori M, Tohidi F, Ghasemikhah R. Serological study of fascioliasis using indirect elisa in Gorgan city, Golestan Province, northern Iran. Iran J Parasitol. 2020; 15(3):418-24. [DOI:10.18502/ijpa.v15i3.4207] [PMID] [PMCID]

[45] Baharsefat M, Massoud J, Mobedi I, Farahnak A, Rokni MB. Seroepidemiology of human hydatidosis in Golestan Province, Iran. Iran J Parasitol. 2007; 2:20-24. [Link]

[46] Fathi S, Ghasemikhah R, Mohammadi R, Tohidi F, Sharbatkhori M. Seroprevalence of hydatidosis in people referring to reference laboratory of Gorgan, Golestan Province, northern Iran 2017. Iran J Parasitol. 2019; 14(3):436-43. [DOI:10.18502/ijpa.v14i3.1483] [PMID] [PMCID]

[47] Ugbomoiko US, Ariza L, Heukelbach J. Parasites of importance for human health in Nigerian dogs: High prevalence and limited knowledge of pet owners. BMC Vet Res. 2008; 4:49. [DOI:10.1186/1746-6148-4-49] [PMID] [PMCID]

[48] Siniscalchi M, d'Ingeo S, Minunno M, Quaranta A. Communication in Dogs. Animals. 2018; 8(8):131. [DOI:10.3390/ani8080131] [PMID] [PMCID]