Seroepidemiology of Human Hydatidosis Using AgB-ELISA Test in Isfahan City and Suburb Areas, Isfahan Province, Central Iran

Parisa ILBEIGI1, Mehdi MOHEBALI1,2, Eshrat Beigom KIA1,2, Mohammad SABER-INASAB1, Mojgan ARYAEIPOUR1, Negar BIZHANI1, *Mohammad Bagher ROKNI1,2

1. Dept. of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Iran
2. Center for Research of Endemic Parasites of Iran (CREPI), Tehran University of Medical Sciences, Tehran, Iran

*Corresponding Author: Tel: 0098-21-88950184 Email: roknimoh@tums.ac.ir

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Abstract

**Background:** The aim of this study was to conduct a sero-epidemiological survey in Isfahan City and suburb areas, central Iran to detect the rate of human hydatidosis using ELISA test.

**Methods:** Overall, 635 serum samples were collected from subjects referred to different health centers in urban and rural regions of the city. Sera were analyzed using Ag-B ELISA test. Ten μg/ml antigens, serum dilutions of 1:500 and conjugate anti-human coombs with 1:10000 dilutions were utilized to perform the test. All subjects filled out a questionnaire and an informed consent. Data analysis was conducted using SPSS 18 software. Cut-off was calculated as X+3 SD.

**Results:** Cut-off value was calculated 0.19. Seven cases (1.1%) were seropositive for hydatidosis by ELISA test. The sero-prevalence of hydatidosis was 0.27% among females and 2.24% among males ($P=0.019$). Age group of 60-69 years old, with 2.59% as prevalence had the highest rate of positivity. There was no significant difference as regards age groups, job, residency, contact by dog and literacy. According to job, self-employed people had the highest rate of infection as 3.05%. The sero-prevalence of infection was 1.14% in diploma and 1.13% in illiterates. As regards residency, urban life (1.49%) showed no significant difference with rural life.

**Conclusion:** The rate of prevalence in this region showed that necessary cautions should be taken into account to monitor the spread of human hydatidosis in this region. In comparison with other studies, the rate of infection was roughly less than other regions.

**Keywords:** Sero-epidemiology, Human hydatidosis, ELISA, Iran

Introduction

One of the most important helminthic diseases is hydatidosis or cystic echinococcosis, established via ingesting parasite’s egg through soil, air, vegetable, contacting with dog etc. (1). Unilocular cyst (produced by *Echinococcus granulosus*) and multilocular cyst (produced by *E. multilocularis*, respectively) are among the most important human diseases in terms of imposed DALYs and economic damages (2, 3). Fashi et al. has estimated the number of asymptomatic individuals living in Iran as 635,232, in addition to overall annual cost of cystic echinococcosis as US$93.39 million (4). As for livestock, the annual cost associated was estimated at US$132 million. The cost per surgical human case was estimated at US$1,539 (4). Iran has been considered as a country of endemic situation by WHO and various researches attested this claim (5-7). So far, many studies have been conducted in different parts of Iran to show the seroprevalence of human hydatidosis. Accordingly,
the rate of prevalence has been reported from 1.2% to 13.7% in different provinces (6, 8-12). Iran has ideal situation in terms of the risk of infecting with hydatidosis such as the high rate of dogs infected with *E. granulosus*, food habit of eating row vegetable, presence of nomads in southern provinces, dusts etc. (5, 6, 13, 14). Hence, it is of great importance to monitor the situation of human hydatidosis regularly throughout the country.

Although many diagnostic tests have been evaluated and established to diagnose hydatidosis, (1, 15-17), but in Iran mostly ELISA test using AgB has been reported as an authentic and valuable test for this issue (6). ELISA has the potential to be served as a vehicle to detect the seroprevalence of some diseases, so it was utilized in this study to detect the hydatidosis in Isfahan Province, central Iran.

The aim of this study was to determine the prevalence of human hydatidosis using ELISA test in Isfahan Province, central Iran.

### Materials and Methods

#### Study Area

Isfahan district is located in central Iran. The city has a population of 2174172 people (Fig. 1). Overall, 635 serum samples were collected from 20 health centers of Isfahan City, and 10 centers from suburb areas. According to the statistician consultant, because population of the city is higher than in rural areas, 468 samples were collected from the city and 167 from rural areas. Based on a random sampling, people were asked to present in health centers for collecting sera.

All participants filled out an informed consent. Besides, a questionnaire including information on food diet, vegetable consumption, clinical symptoms etc. was filled out for each case. In case of inability of the participant to fill out the questionnaire or informed consent form for any reason, his/her guardians were asked to do so.

The study was approved by the Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran.

All sera were examined by ELISA test in Dept. of Medical Parasitology, School of Public Health, Tehran University of Medical Sciences, Iran.

#### Antigen

At first, hydatid cyst fluid (HCF Ag) was aspirated from hydatid cysts obtained from livers and lungs of sheep slaughtered at the local abattoirs of Tehran. Antigen B was purified and extracted as described earlier (18) and was applied in ELISA test.

#### ELISA test

ELISA test was performed in 96 well micro plates (Nunc, Denmark) as previously described (18),
with some modifications. Micro plate wells were coated overnight at 4 °C with 100 μl AgB (10 μg/ml) in 0.05 M bicarbonate buffer, pH 9.6. Wells were washed 3 times in PBS plus 0.05% Tween 20 (PBS-T) and blocked with PBS-T containing 1% BSA for 30 min at 37 °C. Sera were added at 1:500 dilutions in PBST, incubated at 37 °C for 1 h then washed as before. Anti-human IgG-HRP (Sigma Chemical Co., Poole, Dorset, United Kingdom) conjugates were added at 1: 10000 dilutions in PBS-T and the micro plate incubated and washed as before. This was then developed by OPD substrate (5 mg 1, 2-phenylenediamine, 12.5 ml of 0.2 M citrate phosphate buffer pH 5, 10 μl 30% H2O2). The absorbance was read at 492 nm after 10 min using an automatic micro plate reader (State Fax® 2100, Awareness, USA). Altogether 30 samples of sera from healthy volunteers had been collected during the previous studies were examined to set the cut-off.

Data analysis

All data were analyzed using SPSS software ver. 16 (Chicago, IL, USA). P value less than 0.05 was considered as significant. Cut-off was calculated as X+3SD.

Results

The result of seroprevalence study of human hydatidosis was detected as 1.1% (7 cases) by ELISA test in Isfahan Province (Fig. 2). Cut-off was calculated as 0.19, so each OD absorbance higher than this rate was considered positive. The sero-prevalence of hydatidosis was 0.27% among females and 2.24% among males (P=0.019). Age group of 60-69 years old, with 2.59% as prevalence had the highest rate of positivity. There was no significant difference as regards age groups, job, residency, contact by dog and literacy. According to job, self-employed people had the highest rate of infection as 3.05%. The sero-prevalence of infection was 1.14% in diploma and 1.13% in illiterates. As regards residency, urban life (1.49%) showed no significant difference with rural life. Table 1 shows the distribution of age group among cases examined by ELISA test in Isfahan City and suburb areas for hydatidosis.

Fig. 2: Analysis of sera from subjects and normal controls from Isfahan City and suburb areas, Isfahan Province, central Iran by IgG-ELISA using antigen B. Serum samples obtained from subjects (635, Lanes 1), and normal controls (30, Lanes 2)
Table 1: Distribution of positive cases of hydatidosis using ELISA according to age group (yr) in Isfahan City and suburb areas, Isfahan Province, central Iran

| Age group (yr) | Sample (No.) | Positivity (No.) | Sero-Prevalence (%) |
|---------------|--------------|------------------|---------------------|
| 0.0-9.0       | 2            | 0                | 0                   |
| 10-19         | 22           | 0                | 0                   |
| 20-29         | 148          | 1                | 0.67                |
| 30-39         | 123          | 0                | 0                   |
| 40-49         | 122          | 3                | 2.45                |
| 50-59         | 91           | 0                | 0                   |
| 60-69         | 77           | 2                | 2.59                |
| >70           | 50           | 1                | 2.0                 |
| Total         | 635          | 7                | 1.1                 |

Discussion

In this study, we detected a seroprevalence for human hydatidosis using ELISA test in Isfahan City and suburb area, accordingly total prevalence of human hydatidosis was 1.1% (7 cases). Due to long time of prepatent period of human hydatidosis, and the risk of death after consecutive surgeries, it is regarded as one the most important parasitic disease throughout the world. Iran for a long time has been considered as an endemic area for this disease and before considering a watch and wait method to monitor the disease, altogether 1% of all surgeries in Iran belonged to hydatidosis (6, 7, 19). The annual incidence in Iran from various cities are as follows: Hamadan 1.33/100000 (20), Kashan (Isfahan) 3/100000 (8), Babol 1.18/100000 (21) and entire of Iran 0.61/100000 (22).

Many studies have been conducted so far to detect the rate of human hydatidosis in various parts of Iran. Accordingly the rate of infection has been reported as Zanjan 3% (23), nomads tribes from south of Iran 13.7% (24), Ilam 1.2% (25), Kashan 2.04% (8), Sanandaj 7.3% (26), Golestan 2.34% (9), Meshkinshahr 1.79% (10), Tehran 1.63% (27) and Qom 1.6% (28). The rate of infection in our study was 1.1%, which shows more or less a similar rate with other parts of Iran.

In this study, the highest rate of infection was in the age group of 60-69 yr old (2.59%). It is obvious that hydatidosis is a disease of long prepatent period and nearly most cases are diagnosed after a long time, so finding the most cases in higher ages is expectable (29). Previous studies reported the highest rate of infection in 60-90 yr old in Meshkinshahr (10), 30-60 yr in Qomand and Golestan (9, 28), 20-30 yr in Shahryar (12), 40-49 in Arak (11), and > 60 yr in Mongolia (30).

In our study, males showed more seropositivity than females (6/1). Previous studies in Iran shows nearly contradict findings in this regard, which originates from the culture and habit of people in different areas. Some studies have reported more seropositivity in females than males (9, 10, 28, 31), but some studies reported invers results (10, 32) and one study has reported the same findings, i.e., equivalence in males and females (33). Sources of infection with hydatidosis are varies such as soil, contact with dog, eating vegetable, etc., so depending the culture and geographical situation the risk for infection would vary between males and females. For example, in villages where females have more contact with soil and intend to geophagy because of pregnancy, so they encompass more chance to get the disease (5, 6).

Considering occupation, we noticed no obvious difference between seropositive cases, which might be due to low sample, but many studies conducted in Iran and abroad have reported mareseropositivity in housewives and farmers than other jobs (5-7).

In this study, illiterates showed the highest rate of infection (4 cases), which is attested by other studies as well (9, 28, 34). As for residency in the city...
and rural areas, more all seropositive cases belonged to city.
One of the most important limitations of this study might be mentioned as the low rate of sero-positive cases, which is a hinder in statistical analysis, but it would undoubtedly assist for conducting a systematic review and meta-analysis article.

Conclusion

This study would help to complete the puzzle of determining the situation of human hydatidosis in different parts of Iran. Although the rate of infection was not high in Isfahan City in this study, but it should be considered as a challenge in establishing the backbone of healthy measurements.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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References

1. Torgerson PR, Deplazes P (2009). Echinococcosis: diagnosis and diagnostic interpretation in population studies. Trends Parasitol, 25:164-170.
2. Budke CM, Deplazes P,Torgerson PR (2006). Global Socioeconomic Impact of Cystic Echinococcosis. Emerg Infect Dis, 12:296-303.
3. Schurer JM, Rafferty E, Farag M, Zeng W,Jenkins EJ (2015). Echinococcosis: An Economic Evaluation of a Veterinary Public Health Intervention in Rural Canada. PLoS Negl Trop Dis, 9:e0003883.
4. Harandi MF, Budke CM, Rostami S (2012). The monetary burden of cystic echinococcosis in Iran. PLoS Negl Trop Dis, 6.
5. Rokni MB (2008). The present status of human helminthic diseases in Iran. Ann Trop Med Parasitol, 102:283-295.
6. Rokni MB (2009). Echinococcosis /hydatidosis in Iran. Iran J Parasitol, 4:1-16.
7. Sadjadi SM (2006). Present situation of echinococcosis in the Middle East and Arabic North Africa. Parasitol Int, 55 Suppl:S197-202.
8. Arbabi M, Hooshyar H (2006). Survey of echinococcosis and hydatidosis in Kashan region, central Iran. Iran J Peld Health, 35:75-81.
9. Baharsefat M, Massoud J, Mobedi I, Farahnak A, Rokni M (2007). Seroepidemiology of cystic echinococcosis in referred patients to health centers in Golestan Province using ELISA and IFA. Iran J Parasitol, 2 20-24.
10. Heidari Z, Mohebali M, Zarei Z, Aryaipour M, Eshraghian M, Kia E, Shodajei S, Abdi J, Rakhshanpour A,Rokni M (2011). Seroepidemiological study of human hydatidosis in Meshkinshahr district, Ardabil province, Iran. Iran J Parasitol, 6:19.
11. Asghari M, Mohebali M, Eshrat Beigom K, Farahnak A, Aryaeipour M, Asadian S,Rokni MB (2013). Seroepidemiology of Human Hydatidosis Using AgB-ELISA Test in Arak, Central Iran. Iran J Peld Health, 42:391.
12. Sedaghat Gohar H (2012). Seroepidemiologic study of human Hydatidosis in Shahriar area, south of Tehran in 1999. J Kerman Uni Med Sci. 2012; 8 (1): 44-49.
13. Eslami A (1996). Recovery of cestode eggs from the village courtyard soil in Iran. Vet Parasitol, 10:95-96.
14. Eslami A,Hosseini SH (1998). Echinococcus granulosus infection of farm dogs in Iran. Parasitol Res, 84:205-7.
15. Wang LN, Ge LY, Miao F, Yu ZH, Liu YB, Zhen TM, Li GP,Yang SF (2004). [Application of EITB in immunodiagnosis of cisticercosis]. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi, 22:98-100.
16. Kalantari E, Bandchpou M, Pazoki R, Taghipoor-Lailabadi N, Khazan H, Mosaffa N, Nazaripouya MR, Kazemi B (2010). Application of recombinant Echinococcus granulosus antigen B to ELISA kits for
diagnosing hydatidosis Parasitol Res, 106:847-851.

17. Yamano K, Goto A, Miyoshi M, Furuya K, Sawada Y, Sato N (2009). Diagnosis of alveolar echinococcosis using immunoblotting with plural low molecular weight antigens. J Helminthol, 85:57-61.

18. Rokni MB, Aminian B (2006). Evaluation of the Enzyme-linked Immuno-electro Transfer Blot (EITB) technique using hydatid cyst antigens B/5 and total IgG antibodies in laboratory diagnosis of human hydatidosis. Pak J M Sci, 22:127-131.

19. Lotfi M (1999). Hydatid cyst diseases. 1st ed. Sahab Press., Tehran, Iran.

20. Ahmadi NA, Hamidi M (2008). A retrospective analysis of human cystic echinococcosis in Hamedan province, an endemic region of Iran. Ann Trop Med Parasitol, 102:603-609.

21. Ghaffari S (1999). Survey of surgery cases of hydatidosis in three educational-treatment centers of Babol. Journal of Babol University of Medical Sciences 1:27-33.

22. Tavakoli HR, Bahonar AR, Jonidi NA (2008). Epidemiology of hydatidosis in Iran during 2002-2006. Iran J Infect Dis Trop Med, 13:67-71.

23. Haniloo A, Badali H, Esmaeil Zadeh AR (2004). Seroepidemiological study of hydatidosis in Zanjan, Islam-Abad, 2002. Journal of Zanjan University of Medical Sciences & Health Services, 12:41-46.

24. Rafiei A, Hemadi A, Maraghi S, Kalikhaki B, Craig PS (2007). Human cystic echinococcosis in nomads of south-west Islamic Republic of Iran. East Mediterr Health J, 13:41-48.

25. Aflaki A, Gharanjir F, Dalimi Asl A (2005). Seroepidemiological survey of hydatidosis by Dot-ELISA in Ilam province. J Med Sci Modarres, 8:1-6.

26. Akhlaghi L, Massoud J, Housaini A (2005). Observation on hydatid cyst Infection in Kurdestan Province (West of Iran) using epidemiological and seroepidemiological criteria. Iran J Public Health, 34:73-75.

27. Tavalla M, Akhlaghi L, Ourmazdi H, Sarvi S, Razmjoo E, Shokrabi M, Siavoshi M, Beiroomvand M (2010). Using Dot-ELISA Method to Study the Prevalence of Human Hydatidosis in People Referred to Blood Transfusion Center in Tehran, 2005-2006. Razj J Med Sci, 16:52-58.

28. Rakshapur A, Harandi MF, Moazezi S, Rahimi M, Mohebali M, Mowlawi G, Babaei Z, Ariaiepour M, Heidari Z, Rokni M (2012). Seroprevalence of human hydatidosis using ELISA method in Qom province, central Iran. Iran J Parasitol, 7:10.

29. Eckert JD, Deplazes P (2004). Biological, Epidemiological, and Clinical Aspects of Echinococcosis, a Zoonosis of Increasing Concern. Clin Microbiol Rev, 17:107-135.

30. Watson-Jones DL, Craig PS, Badamochir D, Rogan MT, Wen H, Hind B (1997). A pilot, serological survey for cystic echinococcosis in north-western Mongolia. Ann Trop Med Parasitol, 91:173-7.

31. Darani HY, Avijgan M, Karimi K, Manouchehri K, Masood J (2003). Seroepidemiology of hydatid cyst in Chaharmahal va Bakhtiari Province, Iran. Iran J Public Health, 32:31-33.

32. Davami MH, Fatahi Bayat F (1997). An investigation on hydatid cysts which have surgically treated in Markazi Province (Arak). Rahavard Danesh, 5:12-15.

33. Rafiei A, Filip K, Hamzeloei F (2003). Seroepidemiological survey of hydatid cyst in Khuzestan Province. 4th National Congress of Parasitology and Parasitic Diseases, Mashhad University of Medical Sciences pp. p. 48.

34. Qaqish AM, Nasrieh MA, Al-Qaoud KM, Craig PS, Abdel-Hafez SK (2003). The seroprevalences of cystic echinococcosis, and the associated risk factors, in rural-agricultural, bedouin and semi-bedouin communities in Jordan. Ann Trop Med Parasitol, 97:511-20.
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