



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

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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). Fasihi 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 questioner 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.

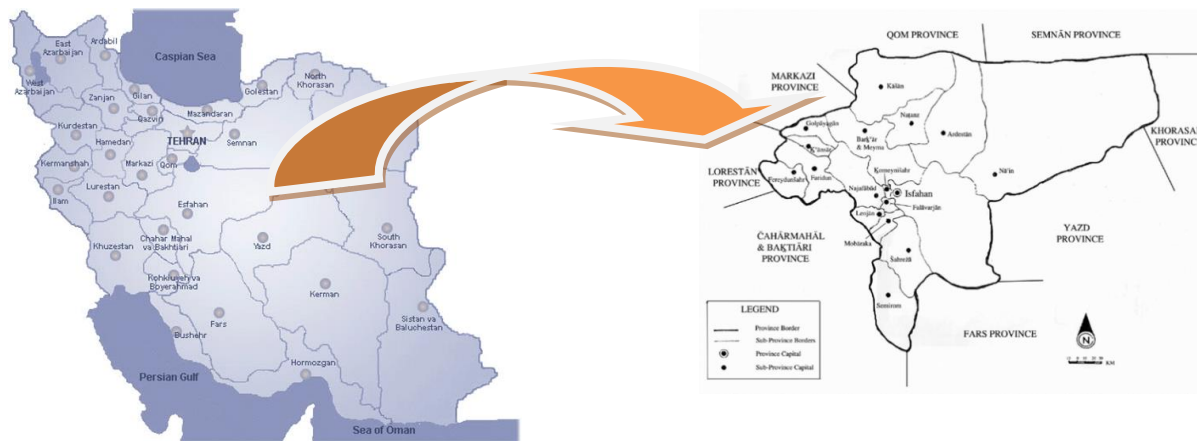


Fig. 1: Location of Isfahan Province in Iran

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. Antihuman 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 text in Isfahan City and suburb areas for hydatidosis.

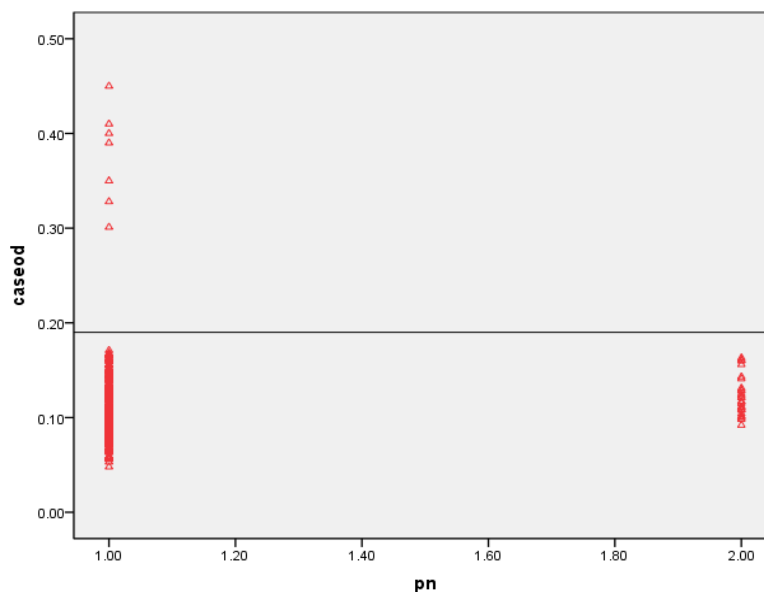


Fig. 2: Analysis of sera from subjects and normal controls from Isfahan City and suburb areas, Isfahan Province, central Iran by IgG-ELISA using antigen B. Serum samples obtained from subjects (635, Lanes 1), and normal controls (30, Lanes 2)

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 more seropositivity 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 seropositive 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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