

Serological Survey of Human Cystic Echinococcosis with ELISA Method and CHF Ag, in Moghan Plain, Ardabil Province, Iran

¹H. Mirzanejadasl, ²M. fasihi harandi and ³P. Deplazes

¹Department of Parasitology, Basic Sciences, Ardabil University of Medical Sciences, Iran

²Department of Parasitology, Kerman University of Medical Sciences, Iran

³Department of Institute Parasitology of Zurich Switzerland

Abstract: Cystic Echinococcosis (CE) is a zoonosis, caused by the metacestode form of *Echinococcus granulosus*. The diagnosis of CE is difficult using the clinical features of the disease and serological methods aimed at determining the specific antibody response. CE is medically and economically one of the important parasitic zoonoses in Iran. So far no survey was conducted to determine the rate of human hydatidosis in Ardabil Province, so using ELISA tests the prevalence of this disease was detected in Moghan area in this Province. Hospital records that the disease is frequent in Moghan area of Ardabil province. The present study designed to determine the seropositive rate and to analyse risk factors of the disease in the people living in the region. In this survey 1998 serum samples were randomly collected from the normal population. The sera was tested using Enzyme Linked Immunosorbent- assay protocol. The serology results All the sera were examined using ELISA test and CFA Ag and Ag B. The serology results were analysed by Logistic regression using spss 11.5. of 1998 serum samples 184 sera (9.2%) were positive. Women were more than men (10% vs 7.9%). The age group of 20-39 and 40-59 showed the highest and the 0-19 showed the lowest rate of infection. The rate of prevalence in this province shows somehow a resemblance with the other cities in Iran. Considering the lifestyle in this province a complementary study is suggested in all related cities.

Key words: Seroepidemiology, human cystic echinococcosis, ELISA, Iran

INTRODUCTION

Cystic Echinococcosis (CE) is a zoonotic helminthiasis of major public health importance in sheep-rearing area worldwide (Eckert and Deplazes 2004), due to its morbidity that causes economic impact and animal production losses and because the affected viscera are condemned at the slaughterhouses. Different assays have been developed and used for the detection of specific antibodies in serum with variable results. However, detection of serum antibody has a major drawback in that demonstration of specific antibodies against hydatid antigen cannot differentiate between recent and past infections (Gottstein *et al.*, 1993). This parasite is cosmopolitan and possesses the second rank in consideration of helminthic diseases significance (Muller, 2002; Torgerson and Budke, 2003; Sadjjadi, 2006). The highest rate of infection is reported from east and south of Europe, Mediterranean coasts, Middle East, Latin America and Africa, mostly in rural districts (Torgerson and Budke, 2003). Larval cysts or hydatid cysts can be found in many

tissues, most often in the liver, lung, mediastinum, peritoneum and nearly every site of the body. CE in the Islamic Republic of Iran is an important but neglected public health and veterinary problem, especially in rural and nomadic communities (Sabbaghian *et al.*, 1975; Nasseh and Khadivi, 1975). Human CE has been reported from different parts of the Islamic Republic of Iran (Sabbaghian *et al.*, 1975; Nasseh and Khadivi, 1975). Saberi *et al.* (1998) reported 13.7% seropositivity in a semi-nomadic community in the country (Saberi *et al.*, 1998). We have reported 9.2% seropositivity in Moghan Plain in Ardabil province. As a rule, sheep act as the intermediate and dogs as the main host of *E. granulosus* in the Islamic Republic of Iran. The prevalence of CE in intermediate hosts from different parts of the country indicates an average rate of about 2-20% (Mohebbali and Sammak, 1995). Half of the Iranian people inhabitants live and work in rural areas as farmers, ranchers and shepherds. Thus CE is a human health hazard and results in economic loss (Rafiei and Craig, 2002). Nonetheless, there have been few sero-epidemiological and mass

screening studies of CE in the Islamic Republic of Iran. Due to the importance of this helminth zoonosis and the lack of information about its prevalence in nomadic communities in the country, we conducted a seroepidemiological study from 2003 - 2005 to assess the prevalence of human CE among Moghan Plain nomads in Ardabil province. We recruited a large sample to determine CE seropositivity and to provide baseline information about the disease prior to the possible implementation of a regional hydatid control programs.

MATERIALS AND METHODS

Ardabil Province is located in northwest Islamic Republic of Iran. This province covers an area of 17953 km² and has a population of approximately 1,168,000; 48% female and 52% male. Moghan area is located in north of Ardabil and 6 area of this Plain is selected. First, 50 Serum samples collected of safety people in Blood transfusion organization of Ardabil for set up of ELISA reader and take cut off. From the selected area, 1998 Serum samples randomly were collected from people of area. A questionnaire was designed to provide the demographic details of the respondents and information about the keeping and handling of dogs and was filled out for each case including various factors such as age, sex, job, locality, literacy, dog ownership, washing vigitable and so on. The questionnaire also enquired about the number of previous surgical operations for CE the respondents had had in the past. Selected individuals questionnaire completed. Each family member was interviewed face-to-face to determine what factor(s) related to their living environment might predispose them to transmission of *E. granulosus*. Venous blood samples (5 mL) were taken from all randomly selected individuals. All blood samples were transferred to the laboratory on ice on the same day of collection and sera were collected and stored at -70°C until tested. Different methods have been used for community screening of CE, but good results have been reported using Enzyme-Linked Immunosorbent Assay (ELISA) methods with an overall sensitivity of 94% and specificity 90% (Craig, 1997). Thus all serum samples were tested for IgG antibodies to *E. granulosus* by microplate ELISA as described previously (Craig, 1997). All data were analyzed using SPSS software ver. 11.5. Hydatid fluid (CHF Ag) was aspirated from hydatid cysts obtained from livers and lungs of sheep slaughtered at the local abattoir. The hydatid fluid was centrifuged at 1000×g for 15 min and supernatant was dialyzed against extensive PBS1X overnight at 4°C. The dialysate was then filtered with Slide-A-Lyser-10K and PBS1X, lyophilized and stored at

-70°C until use in ELISA test. ELISA test was performed in 96 well microplates (Nunc, Maxysorp) as previously described Haniloo *et al.*, (2004) with some modifications. Microplate wells were coated overnight in Humid chamber at 4°C with 100 µL CHFAg (2 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 1:250 dilutions in PBS-T, incubated at 37°C for 1 h then washed as before. Antihuman IgGHRP conjugates were added at 1: 2000 dilutions in PBS-T and the microplate incubated and washed as before. This was then developed in OPD substrate (5 mg l, 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 microplate reader (State Fax® 2100, Awareness, USA). Cut-off was calculated as X + 2 SD.

RESULTS

One hundred eighty four (9.2%) were positive for human hydatidosis in Moghan area in Ardabil Province using ELISA. Results of serological analysis showed that area 4 (Boran and Eivasluo zone) is highest infection and area 6 (Naderkandi and Aghghabagh zone) is lowest infection. There was no significant difference between age groups, sex, Dog owners and occupation, but a significant difference was seen according to place of Dog owners and locality sampling ($p < 0.004$ and $p = 0.000$, respectively). Boran and Eivasluo zone had the highest infection (18.1%). Followed by Naderkandi, Aghghabagh, Aslanduoz, Ider and winter quarters 3.8, 3.8, 8.6, 13.9 and 9.9%, respectively which showed a significant difference ($p = 0.000$). According to place of Dog owners, side of flock had the highest rate on infection (12.3%). Age group of 40-59 yr old had the highest rate of positivity (9.5%). Detailed characteristics of cases according to age group are shown in. Females were more infected than males (10% vs. 7.9%), but the difference was not significant.

DISCUSSION

One hundred eighty four (9.2%) in this study were positive for human hydatidosis using Enzyme-Linked Immunosorbent Assay (ELISA). A study conducted by Saberi *et al.* (1998) reported 13.7% seropositivity in 1000 individuals randomly selected from among Fars nomadic tribes in southern Islamic Republic of Iran (Saberi *et al.*, 1998). Comparing various studies conducted in Iran, more or less, a similar result has been gained. In Urmia, west-Azerbaijan Province, 202 patients with hydatid cyst were detected from 1991 to 2001. 65.85% of

cases were female, whilst farmers and housewives encompassed the highest rate as regards career (Mousavi *et al.*, 2003). In a seroepidemiological study in Zanjan, center of Iran, 3% of studied cases were positive for hydatidosis using indirect ELISA (Haniloo *et al.*, 2004). The highest rate belonged to 10-19 years old. Positive rate in males and females was 2.7 and 3.2%, respectively (Haniloo *et al.*, 2004). The significantly higher rate of CE seropositivity in females in the 15–30 years age group is in agreement with some other epidemiological studies (Shambesh *et al.*, 1999). However, overall, there was no significant association between CE positivity and sex. This contrasts with some previous studies in which the rate of infection was greater in females than males (Hoghoghi, 1971, Shambesh *et al.*, 1999; Macpherson *et al.*, 1998; Abu-Hasan *et al.*, 2002; Abo-Shehadeh, 1993). Some miscellaneous studies reported the prevalence of hydatidosis as 5.9% in Shahryar via IFA technique (Sedaghat Gohar *et al.*, 2001) and 4.8% in Chaharmahal va Bakhtyari Province using counter immunoelectrophoresis (Yousefi *et al.*, 2003). Dogs play an important role in transmitting the disease in Iran. In a widespread study conducted in 13 provinces of Iran, the prevalence of *E. granulosus* in sheepdogs was detected as 27.17% (Eslami and Hosseini, 1998). In addition, in west part of Iran 20% infection rate in stray dogs has been reported (Dalimi *et al.*, 2002). Nine stray dogs (44%) autopsied in Sanandaj were positive for Echinococcosis (Akhlaghi *et al.*, 2005). The reasons for our result could be due to the free movement of dogs among the community and also the lifestyle and culture of the nomads who live in close contact as a big family from their early childhood and so all members are equally at risk of exposure to infection. Our results indicate that there was no significant difference between CE seropositivity and dog ownership. Besides this factor, consuming raw vegetables and contact with soil also have a key factor in acquiring the disease. All these factors can be seen in different area of Moghan Plain. Females were more infected than males (10% vs. 7.9%). Some different studies in Iran showed a higher rate of infection to hydatid disease in females than males (Davami and Fatahi, 1997; Amiri, 2001). More contact with soil and vegetable impose an important factor to cause more infectivity in females. Age group of 40-59 years old had the highest rate of positivity (9.5%). Arbabi *et al.* (1998) reported the highest rate of infection with hydatidosis in Hamadan in age group of 60-80 years (Arbabi *et al.*, 1998) while Hoseini reported this in Kurdistan Province in 20-40 years old (Hosseini, 1997). In this study illiterates showed the highest rate of infection which was in accordance with Sedaghatgohar report in Shahryar district (Sedaghat *et al.*, 2001).

CONCLUSION

In conclusion, our study confirms the high endemicity of human CE in Khuzeestan nomads. Due to the similar living conditions and culture among Iranian nomadic communities and rural areas high CE prevalence is expected to prevail in all such communities also. It is clear therefore that CE is a major public health problem among Iranian nomadic communities and all family members are at the same level of risk of exposure to *E. granulosus* infection.

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