

Serological and Sonographical Survey of Hydatid Disease in Moghan Plain of Iran

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Abstract: Cystic echinococcosis is one of the most geographically widespread Helminth Zoonosis. A study was undertaken to determine spread of Human Cystic Echinococcosis among people in Moghan Plain and to evaluate the effectiveness of health education in increasing community knowledge regarding the disease. Firstly Households were interviewed using a Questionnaire to evaluate their knowledge of Hydatid disease (Cystic Echinococcosis) and the human population was registered and screened for CE by abdominal ultrasound scan as well as a number of serologic tests (ELISA) with HCF-Ag for raw screening and using specific antigens ex. Ag-B and Ag-5 for exact diagnosis. Prevalence of human CE was 9.6% (92 of 1003 sera); 0.3% (8) of the cases were new ultrasound detected asymptomatic cases (mean age = 81 years). Beginning serology results all the sera were examined using ELISA test and HCF-Ag, prevalence of human CE was 11.2% (111), afterwards the serology results all the sera were examined using Specific Antigens Such as Ag-B and Ag-5 for CE prevalence was 9.2% (84 of 1003 sera) this results determined some of the Cysts don't excretion antigen only diagnosed with ultrasound scan. Results were analyzed by Logistic regression using SPSS 13.5. Of 1003 serum samples 84 sera 9.2% were Positive. Women were more than men (20 vs. 11.2%) for CE. The Age group of 1-19 showed the lowest rate and the 20-39 and 40-59 showed the highest rate of infection. Mass screening by US with confirmatory serologic testing is an effective approach to case detection at the everywhere of world.

Key words: Echinococcosis, human hydatidosis, ELISA, Moghan, CE, Iran

INTRODUCTION

Cystic Echinococcosis (CE) is a chronic zoonotic parasitic helminthic disease due to infection of taeniidae family with the larval stage (hydatid) of the dog tapeworm *Echinococcus granulosus*. The parasite has a global distribution but is particularly prevalent in rural areas where it is transmitted in a cycle between the dog, the definitive host and the sheep, the important intermediate host and causing major economically and healthy problems. 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). Human CE is one of the most important endemic infectious diseases in Iran (Muller and Muller, 2002; Torgerson and Budke, 2003; Sadjjadi, 2006). 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. Main clinical symptoms in Humans include Liver dysfunction, lung problems, ascites, abdominal pain,

hepatomegaly, splenomegaly, central nervous system disorders (Muller and Muller, 2002). Cystic Echinococcosis is considered endemic in the entire Mediterranean zone including all countries from the Middle East (Torgerson and Budke, 2003). Both causative agents of the disease are reported in Iran and hydatid disease is responsible for approximately 1% of admission to surgical wards, a figure which has increased remarkably recently due to increasing number of Afghani refugees residing in Iran (Lotfi, 1992; Hadighi *et al.*, 2003). 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). CE has been reported from different parts of the Islamic Republic of Iran (Sabbaghian *et al.*, 1975; Nasseh and Khadivi, 1975; Sharifi, 1997).

In Saberi *et al.* (1998) reported 13.7% seropositivity in a semi-nomadic community in the country. Ultrasonographical and seroprevalence studies in rural communities of Moghan Plain, using ultrasound and an

ELISA with crude sheep hydatid cyst fluid antigen (HCF-Ag) and specific antigens to detect antibodies (total immunoglobulin) revealed an overall Ultrasonographical positive and seropositive rate of 9.6-9.2% (92 and 84 of 1003), respectively. 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% (Mohebali and Sammak, 1995; Haghoghi, 1971). The prevalence of *E. granulosus* infection in dogs has also been reported to range from 3.3-63.3% in sheepdogs (Eslami and Hosseini, 1998). These interpretations showed Iran is one of the importance regions world that *Echinococcus granulosus* spread. Half of the >70 million Iranian 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).

The ability to undertake mass screening programs for human CE has been greatly advanced by the advent of portable ultrasound which has proved highly effective in the diagnosis of asymptomatic cases (Perdomo *et al.*, 1998; Macpherson *et al.*, 1987; Mlika *et al.*, 1986). In addition, serological tests based on Echinococcus antibody detection have also been effectively used in some endemic regions, such as Argentina, to identify asymptomatic seroreactors (Coltorti *et al.*, 1988; Coltorti and Diaz, 1978). However, the combination of ultrasound and serology is likely to be most effective for detection of asymptomatic CE cases (Craig *et al.*, 1996). Nonetheless, there have been few sero-epidemiological and mass screening studies of CE in Iran. Due to the importance of this helminth zoonosis and the lack of information about its prevalence in nomadic communities in this area, we conducted a ultrasound and sero-epidemiological study from 2004-2006 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

The five area of Moghan Plain including Aslandouz, Parsabad, Bilesavar, Boran and winter quarters of Moghan with a population of 208450 persons is located 630 km² from the administrative town of Moghan Plain. On the basis of questionnaires 90% households had owner sheep and domesticated animals nourishment such in winter quarters households used of spring water. With analyzed of questionnaires we known who 64% of

population in this five area consumption without washing vegetables. Ethical approval for the study was given by Ethical Committees of the Ardabil University of Medical Sciences. Informed consent was obtained in writing by participants completing a form detailing he reasons for and procedures used in the proposed community study.

Human screening ultrasound and serologic testing. Families without any age limit and any exclusion factor and living in Moghan villages and winter quarters were assigned a registration number and asked to attend the local medical clinic on a particular day within a scheduled period of 8 days. Individuals were registered by name, age, sex, occupation, Dog owner sheep, knowledge, consumption vegetables and used spring waters and directed to a room where 5 cc of blood were obtained and collected into 15cc ependorph tube. Afterwards stored initially at -20°C and all of sera after 6 days transported to Laboratory center in Ardabil Medicine College and stored at -80°C then antibody testing. Before the collection blood, each individual was directed to a second room for abdominal ultrasound scanning in the horizontal position. Two portable ultrasound scanners with a thermal printout facility (SAL 32- B; Toshiba, Tokyo and Japan) were operated simultaneously by skilled medical ultrasonographer residents. Liver, kidneys, spleen and the upper abdominal area were scanned in each person. Individuals with an ultrasonographic image suggestive of CE or who exhibited any cystic images were asked to donate 5 mL of venous blood for serologic testing. Serum was removed after blood was allowed to clot and separate overnight at 4°C. Initially Serum samples were stored at 20°C then at -80°C before testing. by reason of that 20-25% of cysts not excretion antigens used of ultrasonographic imaging. Cystic images obtained from ultrasound scans of the liver or other sites were graded according to the classification described originally by Gharbi but modified to include an extra category for multiple cysts. Individuals finally diagnosed as having CE were counseled by the local physician and offered treatment that was essentially surgery for patients with viable, noncalcified cysts who were considered operable. Individuals who were seropositive but who had a negative ultrasound result were followed up with a chest radiograph to check for pulmonary cysts and by abdominal computed tomography (CT scan) in two cases. Serologic tests for antibodies to Echinococcus in human sera. Serum samples from those individuals who demonstrated a cyst on ultrasound were tested for total immunoglobulin antibodies by ELISA method and using crude hydatid cyst fluid antigens as previously described (Bonifacino *et al.*, 1991) and for total antibodies using purified antigen B and Ag-5 from hydatid cyst fluid

(Wen and Craig, 1994; Rogan *et al.*, 1991). Data were entered into and most analysis was carried out using the spss ver. 13.5 relative risks were calculated with corresponding 95% confidence intervals and P values to identify risk factors associated with infection. Either the chi-square or Fisher's exact test were used to compute two-tailed P values for independent variables. Chi-square tests for linear trend were used in the determination of linear trends in stratified data. A test for binomial variability was used to analyze the distribution of infections with respect to households.

RESULTS

About 1003 individuals of five area of Moghan Plain (urban population) all residents had an abdominal ultrasound scan. This included 434 males with a mean age of 32 years and a median age of 24 years and 569 females with a mean age of 36 years and a median age of 29 years. Of these samples, 96 (9.8%) exhibited a pathologic lesion on the abdominal ultrasound scan.

Of these 92 persons (9.6%) were finally diagnosed (ultrasonographically and serologically) as having CE (mean age = 47 years, median age = 46, range 6-81). All but two cases were located in the liver; two pulmonary cases of CE was identified a follow-up radiograph. Of the 92 cases with CE detected (41 males and 51 females) three had a history of previous operative removal of a hydatid cyst and therefore were considered recurrences. An additional 16 individuals had a history of confirmed hydatid infection (mean age at the time of the current study was 39 years, median age = 45, range = 79) but had a normal ultrasound scan in the current study. Therefore, taking both new and previous CE cases into account, 9.6% (92 of 1003) of individuals of Moghan Plain were currently infected or had been infected with *E. granulosus*. The age-specific prevalence of newly diagnosed infections within the urban population showed a generally upward trend with age (χ^2 -test for linear trend = 43.64, $p = 1.024$). Age prevalence increased from 0% in the 0-5 years-old age group to 11% in the 65-years-old group. Results of serological analyses showed that area 3 (Bilehsavar) is highest infection (20.1%) and area 2 (Parsabad, Naderkandi and Aghabagh zone) is lowest infection (12.3%). 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). Followed by Aslandouz, winter quarters and Boran (Eivazlou) zone, 1.9, 2.6 and 4.5%, respectively witch showed a significant difference ($p = 0.000$).

According to place of Dog owners, side of flack had the highest rate on infection (12.3%). Age group of 40-59 years old had the highest sero-positivity (20.1%). In this study females were more infected than males (21-11.2%) but the difference was not significant. The distribution of ultrasound images, following essentially the classification of Gharbi and others, for newly diagnosed cysts in both the urban and the rural population samples is shown in Table 1. Ultrasound image classification and frequency of hepatic hydatid cysts in the Moghan Plain and rural population samples tents from the Moghan Plain sample were designated as having CE. When combined with the winter quarters sample, a total of 92 of 1003 sera (9.6%) individuals had an ultrasonographic image highly pathogonomic of CE (Table 2). Fourthly three newly diagnosed CE patients from four area of Moghan Plain and sixteen from the winter quarters were classified by ultrasound as Types I, IV or V. Although suggestive of CE in an endemic region, these could not regard as strictly pathogonomic for *E. granulosus* based on ultrasound image alone. Table 1 summarizes the serologic data broken down by ultrasound image classification for those 111 individuals considered to have CE by ultrasound. Overall 82.3% (94 of 111) were seroreactive tests. For hydatid cysts classified on ultrasonographic images as Types I (simple), IV (solid mass) or V (calcified or partially calcified), 87.8, 80 and 95.5%, respectively were seropositive in one or more tests for an overall rate of 62.5% (63 of 94). The ELISAs using either crude hydatid cyst fluid antigen or purified antigen B and 5 to detect total Immunoglobulines were significantly more sensitive (47.1, 71.6 and 67.4%, respectively). The overall serologic sensitivity for operated CE group was therefore 84.4%. Clinically, a hepatic cyst presenting on ultrasound as a solid mass (Type IV) presents the most difficult diagnostic challenge. Of the 31 CE cases that underwent surgery, seventeen were Type IV and all were seropositive; therefore, serologic confirmation in these patients was important in recommending treatment. At the household level, only single family members were diagnosed with CE in the majority of them (61 cases in 54 households). Of these 61 households 23 separate households don't owned dogs but they consumption wild vegetables without washing and they have direct contact with digs. In this study in defined individuals who washed the wild or eatables vegetables with salt or detergents lowest have infection ($p = 0.003$) and households that washed vegetables with only water or not washed highest have infection ($p = 0.023$). On the basis of questioniers individuals that have knowledge about CE less were than people were not knowledge infection.

Table 1: Ultrasound image classification of hydatid cysts and seroreactivity in Moghan Plain and rural cystic echinococcosis cases ▲

Results/Hepatic cyst classification	Total number of patients		EgCF-ELISA total Ig (%)	Eg-Ag 5-ELISA (%)	Eg-Ag B-ELISA (%)	Total seropositive
	HCF-Ag positive ⑥					
Type I (univesicular)	58		46 (80.0)	51 (86.4)	42 (70.5)	52 (87.8)
Type II (laminated membrane)	8		6 (81.0)	3 (42.0)	2 (25.0)	7 (84.0)
Type III (daughter cysts)	9		7 (80.0)	5 (46.0)	8 (89.2)	6 (70.0)
Type IV (solid mass)	15		13 (63.7)	6 (40.0)	14 (95.0)	12 (80.0)
Type V (calcifications)	11		9 (85.4)	5 (48.3)	8 (72.3)	10 (95.5)
Type VI (multiple cysts)	9		5 (60.0)	3 (38.5)	5 (65.4)	7 (78.0)
Total	111		86 (71.1)	73 (60.4)	78 (66.7)	94 (82.3)

▲ EgCF = *Echinococcus granulosus* Cyst Fluid; EgB = *E. granulosus* B antigen and EgAg5 = *E. granulosus* 5 antigen; ⑥ Moghan Plain and rural cases

Table 2: Positive rates of *E. granulosus* ELISA and ultrasound in various ages of Moghan Plain of Iran

Results/Age groups	No. examined of ELISA	ELISA with Sp.Ag ▲		No. examined of ultrasound	Ultrasound	
		No. positive	%		No. positive	%
5-19	181	3	1.9	181	3	1.9
20-39	192	23	12.3	192	26	14.2
40-59	219	45	20.1	219	47	21.2
60-79	221	6	2.6	221	8	3.1
>80	185	7	4.5	185	8	4.7
Missed	5	-	-	5	-	-
All	1003	84	9.2	1003	92	9.6

▲ Sp. Ag = Specific Antigens ex. Ag-B and Ag-5

DISCUSSION

Human Cystic Echinococcosis (CE) is one of the major zoonotic parasitic diseases in the Middle East and Arabic North Africa from Morocco to Egypt. Both cystic and alveolar echinococcosis has been reported from these areas. However, Cystic Echinococcosis is more prevalent and has been reported from all countries in the Middle East and Arabic North Africa. Alveolar echinococcosis is less prevalent and has been reported only from Iran, Turkey, Iraq and Tunisia (Sadjjadi, 2006). *Echinococcus granulosus* is highly prevalent in Iran, Turkey, Iraq, Morocco, Tunisia and Libya (Azlaf and Dakkak, 2006; Sadjjadi, 2006; Torgerson and Budke, 2003). In the Levant countries, the cystic echinococcosis is also highly endemic. In Oman, it is endemic with low prevalence and a very low level in Cyprus (Sadjjadi, 2006). Various surveys have indicated that hydatid cysts are commonly found in sheep, cattle, goats and camels throughout these regions (Azlaf and Dakkak, 2006; Dalimi *et al.*, 2002; Daryani *et al.*, 2007). Sheep are the most infected animals of these regions. Most of studies on human have been focused on surgical reports although several population studies have been performed using serological and imaging techniques (Frider *et al.*, 2001; Sadjjadi *et al.*, 2001). Present study was carried out to determine serological and sonological methods whether infection with CE represents a public health threat in Moghan areas and to identify regions with high parasite transmission. Present data showed that the highest rate of the infection was in Boran and Eivazlou zone of Moghan Pain and the lowest was Parsabad (Naderkandi and Aghabagh) zone of

the Moghan. A certain number of cases are not seen in hospitals because the infection is asymptomatic or does not require surgical intervention. Secondly, infected dog is the direct or indirect source of infection for humans and therefore, the prevalence of canine infection is one of the most reliable indicators of the potential danger to humans and probable of the canine infection in Moghan Plain of orth west of Iran is more and it is related to suitable geographical conditions. The prevalence and distribution of *Echinococcus granulosus* in sheep dogs was studied in provinces of Iran. Worms were found in 27.17% dogs. The highest prevalence was detected in dogs from the rural areas of Esfahan Province (central part of Iran) and the lowest in dogs from those of Sistan and Balochestan Province (Southeast Iran) (Eslami and Hosseini, 1998). Also in Fars Province, one hundred and five stray male and female dogs in different age groups were autopsied and their small intestines examined for *Echinococcus granulosus*. Thirty eight dogs (36.19%) harboured two-several thousand *E. granulosus* in their intestinal content (Mehrabani *et al.*, 1999). In the other hand, infection was seen in other regions of Iran but CE in Ardabil Province specially is endemic. The serological and sonological prevalence of human CE was reported in 5 areas of Ardabil Province (Moghan Plain) was 1.9% (Aslandouz, 4.5% (winter quarters), 12.3% (Parsabad (Naderkandi, Aghabagh)), 20.1% (Bilesavar) and 2.6% (Boran) (Eivazlou zone) (Table 3). In this study, female patients were more than male. This is similar to othe studies in Iran (Mamismi *et al.*, 2007) in the UK, the Middle East and North Africa (Torgerson and Budke, 2003). This may reflect that women were more likely to

Table 3: Prevalence of CE in areas of Moghan Plain, North West of Iran

Results/ Area	No. examined of ELISA	ELISA Sp.Ag No. positive (%)	No. examined of ultrasound	ELISA HCF-ag No. positive (%)	Ultrasound No. positive (%)
Area 1: Aslandouz	181	3.0	181	3.00	3.0
	-	1.9	-	1.90	1.9
Area 2: Parsabad	192	23.0	192	36.00	26.0
(Naderkandi and Aghabagh zone)	-	12.3	-	17.40	14.2
Area 3: Bilesavar	219	45.0	219	54.00	47.0
	-	20.1	-	24.30	21.2
Area 4: Boran and Eivazlou zone	221	6.0	221	7.00	8.0
	-	2.6	-	2.67	3.1
Area 5: Winter quarters	185	7.0	185	11.00	8.0
	-	4.5	-	6.00	4.7
Missed	5	-	5	-	-
	-	-	-	-	-
All	1003	84.0	1003	111.00	92.0
	-	9.2	-	11.20	9.6

seek treatment. The highest rate of human CE was showed in age: 40-59 years old. Bastani and Dehdashti (1995) also showed 60% of the patients were in the third and fourth decades of life. In study of Torgerson and Budke, 2003; analysis of data suggested that the likelihood of an affected patient having a cyst decreased with age. In the present study, cysts were found in 73.8% cases in liver in 17.7% cases in lung, while they were also found in the spleen (2.5%), abdomen (1%), brain (1.5%), both liver and lung (1%) and other organs (2.5%). So, liver and lungs account for >90% (91.5%) of organ involvements and the most hydatid cysts were found in liver and lung, while they were also found in the spleen, abdomen, brain and other organs (Sadjjadi, 2006). Splenic involvement is rare occurrence of about 2.5% and in another study were reported two cases of hydatid disease of the spleen in Izeh, a city in Khuzestan Province of Iran (Azordegan *et al.*, 2007). Mamishi *et al.* (2007) showed that cysts found in the lungs and livers of 24 (77%) and 15 cases (48%), respectively with 8 cases (26%) having simultaneous liver and lung cysts. Three patients (10%) had multiorgan involvement. Meanwhile, cyst may find in unusual location. For example in an Iranian Moslem patient cyst was found in ovary (Azhar, 1997). The striking clinical resemblance between hydatid disease and malignant diseases of some organs makes the correct diagnosis essential. In countries where this disease is endemic, any growing mass or tumour should arouse suspicion of hydatid disease (Emamy and Asadian, 1976).

CONCLUSION

This study proved highly useful in determining the incidence of hospitalized CE cases and these findings indicate that CE is a public health and an economic problem in Iran especially in Moghan Plain of Ardabil Province. There are reasonable grounds for presence of the infection. The infection in stray dogs and other carnivores, bad abattoirs, the frequent slaughter of

animals in places other than abattoirs and illegal immigrations especially in eastern border of the country appear to be the main reasons for the high prevalence of echinococcosis in Iran.

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