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Serological evidence of human cystic echinococcosis and associated risk factors among general population in Mazandaran Province, northern Iran

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HIGHLIGHTS

- This article reported new seroprevalence information on the present situation of the human cystic echinococcosis (CE) in Mazandaran Province, northern Iran.
- The results indicated the high seroprevalence of CE especially in rural areas.
- Our study also shows that consuming of the raw vegetables, contact with dog and soil are as main risk factors for this disease.

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ABSTRACT

The aim of the present study was to determine the seroprevalence of CE among human referring to Health Centers in Mazandaran Province, northern Iran and to identify the risk factors involved in spreading the disease. Between 2013 and 2014, the serum samples were taken randomly from 600 subjects referring to health centers in Mazandaran Province. After obtaining informed consent for each participant, a questionnaire including demographic characteristics and associated risk factors was filled for each individual. Anti-CE antibody was tested by enzyme-linked immunosorbent assay (ELISA), using native antigen B. Our results showed 31.6% (n = 190) seropositivity. There were significant difference between seropositivity and sex and residence. Males were significantly more seropositive than females (24.6% versus 7%, P = 0.0001). Regression analysis showed that the subjects who are living in rural areas were 4.4 times more likely to be at risk to CE than urban areas (OR = 4.4; 95% CI = 2.91, 6.64). Contact with dogs, soil and consumed raw vegetables was appeared as main risk factors for CE among community in Mazandaran and it may increase the probability of infection. The high prevalence of CE among individuals indicated that hydatidosis is still a major health problem among community in the investigated areas.

1. Introduction

Cystic echinococcosis (CE) or hydatidosis is a zoonotic parasitic disease caused by the larval stages of tapeworms belonging to the genus *Echinococcus* between human and livestock. Hydatidosis is also considered as the most fatal and important helminthic diseases in Iran and throughout the world [1–3]. Dog as definitive host of *Echinococcus granulosus* (*E. granulosus*) assume the most important role in scattering infection in the Middle East countries including Iran. The burden of CE causes remarkable economic losses and health public problems in domestic animals and human [4,5]. The disease has significant economic impacts with the overall annual cost estimated about at US $ 232.2 million of the gross domestic products [6]. In addition, numerous livestock have been commonly found infected with CE in Iran [7,8].

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Diagnosis of human hydatid cyst (HC) by using of serological techniques is useful for approval of CE, as well as discriminate it from other cystic lesions in endemic areas [9,10]. Moreover, serological tests are valuable for the follow-up of patients after surgical or treatment all through epidemiological investigations [11,12].

High prevalence of *E. granulosus* was reported from East and North Africa, Mediterranean countries, Middle East [8,11]. Iran is regarded as an endemic region of hydatidosis and the disease has been reported quite throughout the country. In most areas of Iran, the prevalence of human hydatidosis has estimated 0.6–1.2/100000 population [2,13].

To the best of our knowledge, no study has been performed to determine seroprevalence of CE among human general population in Mazandaran Province, northern Iran, up to now. On the other hand, little is known regarding the prevalence, incidence, and true burden of CE public health within endemic communities in Iran [6]. Therefore, the goal of the present study was to determine the seroprevalence and associated risk factors among individuals attending to Health Centers, for the first time, in Mazandaran Province, northern Iran.

2. Materials and methods

2.1. Study area

The current study was performed in Mazandaran Province, north of Iran, from September 2013 to March 2014. Mazandarn Province is located in northern of Iran (53°6′ E, 36°23′ N), in the south of Caspian Sea, with subtropical climate conditions. According to the census of 2004, the population of the province was about 5,000,000 million of which 46% were registered as urban dwellers, 54% dwell in the rural areas and the remaining were non-residents, and the climate of the study area is sub humid to humid, while rainfall ranges are from 718 to 1274 mm annually [14].

2.2. Sample collection

Five ml blood sample were collected randomly from volunteers attending to the public and private health centers in Mazandaran Province throughout 2013–2014. Sera were separated and stored until use at −20 °C. After obtaining informed consent for each participant, a questionnaire including sex, age, area of living data, socio-demographic characteristics, and associated risk factors (education level, occupation, consumed raw vegetables, contact with dog and soil) was filled out for each individual. The Ethics Committee of Mazandaran University of Medical Sciences gave ethical approval of the study and consent was achieved from the participants.

2.3. Antigen preparation and testing

For preparation of antigen B (AgB); crude hydatid cyst fluid (HCF) was collected from infected livers of sheep slaughtered in local abattoirs of Mazandaran. Briefly, at first 100 ml of fresh HCF was centrifuged at 1000*g* for 15 min and then it was dialysed overnight using dialysis cassettes against 0.005 M acetate buffer (pH 5.0) at 4°C. The HCF was centrifuged at 50000*g* for 30 min and the precipitate was dissolved in 0.2 M phosphate buffer (pH 8.0). Saturated ammonium sulphate was used to remove the globulin fraction from the HCF. Next, the purified HCF antigen was boiled at 100 °C for 15 min in a water bath and centrifuged at 50000*g* for 60 min to separate Ag B. The protein concentration of sample was measured and the antigen was kept at −20 °C until use [15]. The 5 μg/ml of purified Ag B (100 μl/well) was coated in 96-well ELISA (Enzyme-linked Immunisorbent Assay) microplates (NUNC, Denmark) and kept at −20 °C until use. IgG-ELISA test was carried out on serum samples taken from study population as described by Sarkari et al. [16] The optical density (OD) at 490 nm was measured using an ELISA plate reader. The cut-off value was determined as the mean plus two standard divisions (2SD) of the OD observed with apparently healthy subjects as normal controls.

2.4. Statistical analysis

A Chi-square test was used to determine the significance in prevalence according to the variables. Odds ratios and confidence interval for risk factors analysis were calculated by logistic regression model forward method. All statistical analyses were carried out using the SPSS version 16.0 software. P-value < 0.05 was considered significant.

3. Results

In the present study, of 600 subjects examined, 383(63.8%) were males and 217(36.2%) females. Also among them 356 (59%) were living in rural areas and 244 (41%) in urban areas. Serological results showed 31.6% (n = 190) seropositivity. Males were significantly more seropositive than females (24.6% versus 7%, P = 0.0001). In addition, logistic regression analysis showed that males were 2.6 times more likely to be seropositive than females (OR = 2.6; 95% CI = 1.77, 3.89) (Table 1).

In view of the subject's residency, the highest rate of infection was 25.7% in rural and the lowest was 6% in urban areas. Regression analysis showed that subjects who are living in rural areas were 4.4 times more likely to be at risk to CE than urban (OR = 4.4; 95% CI = 2.91, 6.64) (Table 1).

Data analysis showed that educated subjects were 1.5 times more likely to be at risk than non-educated (OR = 1.5; 95% CI = 0.98, 2.31), while the differences were not significant (P = 0.06). According to occupation, the highest and lowest rate of infection were found among other (10.7%) job and farmers 2.3%, respectively (Table 1) and no statistical significant differences were observed among different jobs (P > 0.05). Additionally, the regions with the highest and lowest prevalence rates of CE were Babol (17.8%) and Neka (0.15%) respectively (Table 2).

Moreover, logistic regression results showed subjects who had contact with dog, soil and consumed raw vegetables 15.4 times and those had contact with dog and no contact with soil 2.5 times more likely be at higher risk of infection than who had no contact with dog and soil (Table 3).

4. Discussion

Although CE is one of the most important zoonotic parasitic diseases in Iran and is endemic in most parts of the country, but little information is available concerning the epidemiology of the disease and its public health importance in some parts of Iran. The present study showed a high (31.6%) seroprevalence of CE as well as the association risk factor with infection, as the first community-based survey among general population in Mazandaran Province.

Serologic tests are the most widely used methods, one that is applicable, low-cost, not time consuming, and easy to perform on large numbers of serum samples. Previous studies in different parts of Iran shows the rate of disease from 1.2% to 21.4%, based on serological methods mainly ELISA [2,10].

In some of the studies conducted in different regions of Iran, the seroprevalence of hydatidosis in human have been reported by researchers from Qom Province as 1.6%, Yasuj 7.2%, Zanjan 3%, Kashan 2.4%, Meshkin Shahr 1.8%, Ilam 1.2%, Golestan Province 2.3% and Khuzestan 13.8% [10,16–22]. However, the seropositivity rate

Table 1
Logistic regression analysis of seropositive cases of hydatid cyst according to sex, age, residence, education, occupation, use vegetable, contact with soil and contact with dog, in Mazandaran Province, Iran.

Variables		Sero-prevalence			
		No. of Examined (%)	No. of Positive (%)	Odds Ratio (95% CI[a])	P-value
Sex	Male	383(63.8)	148(24.6)	Reference[b]	P < 0.0001
	Female	217(36.2)	42(7)	2.62(1.77, 3.89)	
Age(Year)	15–30	108(18)	35(5.8)	Reference	–
	30–60	442(73.7)	134(22.3)	1.10(0.70, 1.73)	0.67
	≥60	50(18.3)	21(3.5)	0.66(0.33, 1.32)	0.24
Residence	Urban	244(40.7)	36(6)	Reference	P < 0.0001
	Rural	356(59.3)	154(25.7)	4.40(2.91, 6.64)	
Education	Educated[c]	461(76.8)	155(25.8)	Reference	0.06
	Non-educated	139(23.2)	35(5.8)	1.51(0.98, 2.31)	
Occupation	Employed	93(15.5)	27(4.5)	Reference	–
	Greengrocer	200(33.3)	44(7.3)	0.69(0.39, 1.20)	0.19
	Rancher	20(3.4)	17(2.9)	13.85(3.75, 51.16)	0. 33
	Farmer	18(3)	14(2.3)	8.58(2.58, 28.35)	0. 41
	Housekeeper	90(15)	24(4)	0.89(0.47, 1.69)	0.72
	Other	238(39.7)	64(10.7)	1.36(0.79, 2.34)	0.27
Consumed raw vegetables	NO	22(3.7)	3(0.5)	Reference	0.04
	Yes	578(96.3)	187(31.2)	3.02(0.88, 10.36)	
Contact with soil	NO	248(41.3)	30(5)	Reference	P < 0.0001
	Yes	352(58.7)	160(26.7)	6.06(3.90, 9.40)	
Contact with dog	NO	400(66.7)	64(10.7)	Reference	P < 0.0001
	Yes	200(33.3)	126(21)	8.94(6.04, 13.23)	
Total		600(100)	190(31.6)	–	–

[a] CI: confidence interval.
[b] Reference: The level of variable which other levels compared with it.
[c] Educated: defines as illiterate, primary, secondary school, college and above.

Table 2
Distribution of seropositive cases of hydatid cyst in residential areas in different township in Mazandaran Province, Iran.

Location	Sero-prevalence		
	Positive cases no (%)	Negative cases no (%)	Total no (%)
Neka	1(0.15)	20(3.3)	21(3.5)
Sari	24(4)	79(13.2)	103(17.2)
Ghaemshahr	20(3.3)	48(8)	68(11.3)
Babol	107(17.8)	114(19)	221(36.8)
Babolsar-Amircola	10(1.6)	32(5.3)	42(7.5)
Polasafid-Zirab	16(2.6)	57(9.5)	73(12.2)
Amol	2(0.3)	15(2.5)	17(2.8)
Tonekabon	4(0.6)	20(3.3)	24(4)
Ramsar	6(1)	25(4.2)	31(5.2)
Total	190(31.6)	410(68.4)	600(100)

Pearson Chi-square = 56.39, df = 11, P < 0.00001.

in our study was higher than that to be found in about all reports from Iran and than that found in other countries [2,23–26] which considering the conditions of the studied area in our work was expectable in Mazandran Province.

In some districts of Iran, particularly in the north, where a large population of dogs, jackals, and foxes are living neighboring to human settlements, the interaction between domestic and wild cycles of *E. granulosus* may be occurred [17,27,28]. In addition, Mazandaran Province climatically represents a more favorable environment for the survival of *Echinococcus* eggs. Dogs are the most successful animal adapted to human residence worldwide [29–31]. They have donated to physical, social, and emotional happiness of their owners, chiefly children who are frequently at maximum risk of exposure. In appropriate human practices and behavior and closely communication to dogs may remain as a main danger to public health [31,32]. On the other hand, a recent study in slaughtered herbivores in Mazandaran demonstrated the situation of CE infection in livestock as followed 65.2%, 37.8% and 40.1% in sheep, goats and cattle; respectively [33]. These data confirms the high infection rate of CE among domestic animals in the area.

Our study showed that males were significantly more seropositive than females (24.6% versus 7%). This may be due to the local culture and behavioral tasks of the men such as farming, herding and feeding dogs, where those had more contact with environmental risk factors than women. Although the most studies in Iran and other countries indicated that, seropositivity in females were more than males [1,17,25,34,35]. In contrast, recently Zibaei et al. [36] have reported that males were significantly more seropositive than females in a district in western Iran. We suggest that this

Table 3
Logistic regression analysis of seropositive cases of hydatid cyst according to the contact with dog, soil and consumed raw vegetables in Mazandaran Province, Iran.

Risk factor	Sero-prevalence				
	Positive cases n(%)	Negative cases n(%)	Total n(%)	Odds Ratio (95% CI*)	P-v**
No contact with dog and soil	20(3.3)	180(30)	200(33.3)	Reference***	–
Contact with dog, soil and consumed raw vegetables	126(21)	74(12.3)	200(33.3)	15.4(8.9, 26.3)	P < 0.0001
Contact with dog and No contact with soil	44(7.3)	156(26)	200(33.3)	2.5(1.4, 4.5)	0.001

CI: confidence interval **P-v: P-value *** Reference: The level of variable which other * levels compared with it.

difference should be studied in the different cultural and environmental conditions in the further.

In our study, the seropositivity of CE in the different age group was not significant. Our analysis showed that the 30–60 years age group had a more chance of exposing to *E. granulosus* egg than younger and older age groups. Additionally, the older age groups (60 and older) had lower rates of seropositivity than the younger ones. Other studies showed CE is the most prevalent relatively in middle ages (30–40 years) of life [1,16,18,37]. Lower mobility and low immunological response in older peoples may be the reason for lack of seropositivity in older age groups. The highest number of CE cases were recorded by age groups: 21–30 years in Kenya, 21–40 years in Libya and 30–39 years in Turkey, 20–39 years in Iran [11,25,34]. In general, several researchers reported that the seropositivity rate for CE increased significantly with increasing age [16,38,39].

Our study, showed no significant correlation with educational status, although the prevalence for CE in educated individuals was higher (almost 1.5 times) than non-educated one. ($P = 0.06$). It might be occurred due to lack of equilibrium ratio of both groups (educated/non-educated ratio, 3.3:1) in the study.

Residential areas in the Mazandaran Province especially living in rural areas are another risk factor recognized in our study. The results of our study showed 25.6% seropositive subjects living in rural and 6% in urban areas. This means that the risk of disease is four times higher in rural than urban areas. Our results are similar to the previous studies where investigated in other countries including Iran [6,18,25,33,34].

Our analysis, showed no significant correlation with occupation, but the prevalence for CE in other occupational groups (10.2%) was higher than in the rest of one. ($P = 0.27$).

Regarding the prevalence of CE in the nine townships screened, the highest and lowest prevalence rates were detected in Babol (located in central part) and Neka (located in eastern part), respectively. There were statistically significant differences between townships in different part of the province. Thus, the highest seroprevalence of hydatid cyst was found in central parts of Mazandaran Province. The reason of this condition is unknown, thus more investigations among high-risk groups are recommended in the part of the province.

The results of our study showed that contact with dogs, soil and consumed raw vegetables statistically associated with the rate of infection. This is agreement with most other studies carried out in Iran and other countries [18,40,41]. Therefore, it seems that these behaviors were appeared as main risk factors for CE in the region (Table 1) and it may be increased the probability of infection rate. Consequently, contact with dogs and consumed raw vegetables could be play an important role in transmitting the disease to all people at risk of exposure to infection in north of Iran.

However, the present study has some limitations. First of all is the absence of a confirmatory test that would enable evaluation of alternative diagnostic tests. However, the ELISA we used is one of the most sensitive serological tests for the diagnosis of hydatid disease, and is inexpensive and relatively easy to use. Moreover, this test can be use for large-scale screening of populations in which hydatidosis is endemic. The presence of a specific antibody alone does not confirm diagnosis, as individuals may be seropositive for a number of reasons, such as previous exposure to the parasite without progressive disease or cross-reactivity with other conditions.

The second limitation is false-positive results, which occur due to cross-reactions with other helminthic disease, such as taeniasis, fascioliosis and toxocariasis [5], which these are common in Mazandarn Province than other regions of Iran [42]; and or non-infectious conditions, such as cancer, pregnancy and autoimmune diseases [23,25]. The third limitation of the study is an imbalance of sexes in the study (male/female ratio, 1.8: 1). However, the sample size for women is statistically conclusive.

All together, the results of our study indicated the high seroprevalence of human cystic hydatidosis in Mazandaran Province, Iran. The high CE prevalence in rural areas is a major public health problem among the Province communities and all individuals are at the risk of exposure to *E. graulosus* infection. This study also showed that consuming of the row vegetables and contact with dogs is associated to the prevalence rate of CE and introduces vegetables as a risk factor for this disease.

In conclusion, the high prevalence of CE among individuals show that hydatidosis is still a major health problem in the investigated areas and will pose a risk for rural inhabitants there. Given data offers mass screening of individuals, particularly at high-risk group and also increase the awareness of general population regarding modes of transmission of this disease. In general, our data provide valuable information concerning the epidemiology of hydatidosis in the northern region of Iran, which will likely be very significant for management and prevention plans of this disease. Health education, proper disposal of infected offal should be considered as the main control measurements to reduce the rate of CE in human and livestock in this CE-endemic area. Moreover, further studies are required to find the reasons for the high rate of seropositivity in the community.

Ethical approval

Mazandaran University of Medical Sciences.

Funding

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Author contribution

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Conflicts of interest

All authors declare that they have no conflicts of interest.

Guarantor

Dr. Mahdi Fakhar.

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