



## 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.

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### 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. Mazandaran 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  $-20^{\circ}\text{C}$ . 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 1000g for 15 min and then it was dialysed overnight using dialysis cassettes against 0.005 M acetate buffer (pH 5.0) at  $4^{\circ}\text{C}$ . The HCF was centrifuged at 50000g 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  $100^{\circ}\text{C}$  for 15 min in a water bath and centrifuged at 50000g for 60 min to separate Ag B. The protein concentration of sample was measured and the antigen was kept at  $-20^{\circ}\text{C}$  until use [15]. The 5  $\mu\text{g}/\text{ml}$  of purified Ag B (100  $\mu\text{l}/\text{well}$ ) was coated in 96-well ELISA (Enzyme-linked Immunosorbent Assay) microplates (NUNC,

Denmark) and kept at  $-20^{\circ}\text{C}$  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

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