

Cystic echinococcosis is an occupational disease?

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Abstract Cystic echinococcosis (CE) is considered as an occupational infection and among different careers greengroceries have a close contact with raw vegetables which can transmit eggs of *Echinococcus granulosus*. Therefore, the objective of this survey was to determine the seroprevalence of CE among greengroceries using ELISA test in Mazandaran and Gilan Provinces, northern Iran. Overall, 160 serum samples (60 male and 100 female) were collected from greengroceries in Mazandaran and Gilan Provinces, 2012. Sera were surveyed employing ELISA assay for diagnosis of CE. In addition, before sampling, a questionnaire was filled out for each subject. Seropositivity was 2.4 % (four cases) and females 1.8 % (three cases) were more positive compared to males 0.8 % (one case). Age group of 40–60 years encompassed the highest rate of positivity (1.8 %) and the least rate was in 20–40 years old age group (0 %). Besides, 1.8 % of seropositive subjects were dog owner, unaware of the disease and lives in rural areas. In conclusion, seroprevalence of cystic hydatid disease among greengroceries is relatively high in

Mazandaran and Gilan Provinces. More continuous serological researches and preventive measures should be taken into consideration owing to the significance of the disease.

Keywords Cystic echinococcosis · Cestoda · Serology · ELISA · Epidemiology · Disease · Iran

Introduction

Cystic hydatid disease (CHD) caused by the larval stage or metacestode of *Echinococcus granulosus* which is cosmopolitan parasite and it is found at least in 100 countries (Eckert and Deplazes 2004). CHD is a matter of high importance not only in medical field due to public health problem but also in veterinary field owing to considerable economic damages and losses including huge waste of animal proteins. Hydatidosis is in the second rank in term of helminthic disease significance (Torgerson and Deplazes 2009).

CHD is considered by the World Health Organisation (WHO) as: “... not only one of the most widespread parasitic diseases, but also one of the most costly to treat and prevent in terms of public health” (Eckert et al. 2001). In addition, cystic echinococcosis (CE) is considered as one of major Neglected Zoonotic Diseases (NZD) by WHO. The annual incidence rate of CHD can vary from 1 to 200 per 100,000 inhabitants in various endemic regions (Silva 2010). The highest rate of CHD is observed from Africa, China, east, and south of Europe, Mediterranean coasts, Middle East, South America and mostly in rural regions (Budke et al. 2006). CE is endemic in the entire Mediterranean area including Middle East countries. Iran is considered either an endemic or hyperendemic area. Human hydatidosis is regarded as a public concern in different parts of Iran. The disease is considered responsible for approximately 1 % of admission to surgical

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wards and infection rate in human is 0.6–1.2/100,000. Hydatid cyst can be observed in all parts of human body. The most involved organ is liver which is followed by lung (Rakhshanpour et al. 2012). Canids are definitive hosts of *E. granulosus* whereas human, sheep and other mammalian species may serve as intermediate hosts. Humans acquire infection by accidental ingestion of eggs of *E. granulosus* via major sources of infection such as soil, vegetables, and association with dogs (Rahimi et al. 2011).

Fresh vegetables are an important part of a healthy diet and raw vegetables can be agent of transmission of parasites to humans. *E. granulosus* eggs were observed in vegetables including lettuce, coriander, chamomile, basil, parsley, watercress and radishes. Coming and going dogs across the vegetable farms help to spread this parasite (Adamu et al. 2012).

Besides, certain people including shepherds, slaughters, stockbreeders, farmers, butchers, veterinarians, tanners and all whose career makes them to work closely with animals are at higher risk of the disease (Farahmand and Yadollahi 2010). Among occupations vegetable sellers are important group who are at high risk of acquiring the infection because of their close contact with raw vegetables which can transmit *E. granulosus* eggs. To best of our knowledge, albeit many efforts have been made to determine the seroprevalence of CHD in general population and different occupational groups, no attention has been paid to greengrocers who sell and distribute raw vegetables. Therefore, the objective of the present study was to determine the seroprevalence rate of infection in greengrocers and also evaluation of their occupation risk factors.

Materials and methods

Study area

Mazandaran (36°33'56"N 53°03'32"E) and Gilan Province (37°27'74"N 49°58'90"E) are located at the northern part of Iran and on the southern coast of the Caspian Sea. Totally they cover an area of 37,884 km² and 32 Counties with a population composed by 5,327,293 inhabitants and a humid subtropical climate with the heaviest rainfall rate in Iran and an average temperature of 25.5 °C in summer and 8 °C in winter. These provinces are geographically divided into the coastal plains and the mountainous areas of Alborz Mountains Range. The areas have diverse ecosystems, including many plains, prairies and forests (Fig. 1; Yossefi et al. 2014).

Sampling

Initially, a questionnaire was filled out for each subject to record information including sex, age, residence place, way

of vegetable consumption, people knowledge of the disease and dog possess after getting informed consent.

Overall, 160 serum samples (60 male and 100 female) were collected from greengroceries in two Provinces (Mazandaran and Gilan), north of Iran, 2012. The blood sample was taken from each individual and transferred to the laboratory of Young Researchers Club, Islamic Azad University Babol-Branch, Mazandaran Province, Iran, for examination with ELISA.

ELISA test

Microplate wells were coated over night at temperature of 4 °C with 100 µl AgB (20 µg/ml) in 0.05 M bicarbonate buffer considering pH 9.6. Wells were washed three times in PBS plus 0.05 % Tween 20 (PBS-T) and blocked with PBS-T containing 1 % BSA for 30 min at 37 °C. Serum samples were added at 1:500 dilutions in PBST, incubated at 37 °C for 60 min afterward washed as before. Antihuman IgG-HRP (Sigma Chemical Co., Poole, Dorset, United Kingdom) conjugates were added at 1:10,000 dilutions in PBS-T and the microplate incubated and washed as before. This was then developed in 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 microplate reader (State Fax[®] 2100, Awareness, USA).

Results

Serological results showed 2.4 % (four cases) seropositivity. Infection rate in females were higher than males 1.8 versus 0.6 % (three females and one male). 1.8 % of infected cases dwell in rural areas compared to patients in urban areas 0.6 %. And a relationship was observed between positive subjects and lack of knowledge of the disease; since all of positive subjects did not have any knowledge of the disease. Whereas, no association was seen between infected cases and vegetable consumption (washed or unwashed). With regard to age, the highest infection rate was seen in 40–60 years age group 1.8 % and the least rate was in 20–40 year old age group as 0 %. Results showed that seropositive people who were dog owner were three times at higher risk for infection compared to people without dog. In addition to, the examined individuals had no history of hydatid cyst surgery and just 3 % of them were used gloves during work. Table 1 depicts the result of examined individuals and their risk factor information.

Discussion

Vegetables play a major role as a significant source for human parasitic infections. CE is a vegetable-borne



Fig. 1 Status of Mazandaran (*green*) and Gilan (*red*) Prov. (Color figure online)

Table 1 Sex, age, residence place, vegetable consumption, knowledge of the disease, dog owner and hydatidosis seropositivity of examined individuals in Mazandaran and Gilan Provinces, 2012

Total number	Sex		Age group (year)			Residence area		Vegetable consumption		Knowledge of the disease		Dog owner	Positive case
	Male	Female	20–40	40–60	60–80	Urban	Rural	Washed	Unwashed	Aware	Unaware		
160 (%)	60	100	47	23	90	65	95	107	53	8	152	18	4
	37.5	62.5	29.3	14.4	56.3	40.6	59.4	66.9	33.1	5	95	11.2	2.4
Positive case (%)	1	3	0	3	1	1	3	2	2	0	4	3	4
	25	75	0	75	25	25	75	50	50	0	100	75	100

parasite in which parasite eggs are considered as only source of infection. It seems that certain occupations are more at danger of the disease because of more exposure. There is a relationship between career and hydatidosis infection. Among different occupations greengroceries are considered as an important job due to being in close contact

with vegetables (Van Duyan and Pivonka 2000; Anuar and Ramachandran 1997).

In the current study, 2.4 % cases were infected by hydatidosis that were in accordance with a variety of different surveys in different parts of Iran (Rokni 2009). Hydatidosis rate in Iran ranging from 1.2 to 21.4 %

according to serological assays mostly ELISA (Rokni 2009). The sero-epidemiology of CHD in humans in different parts of country were reported as the following: Nomads of Khuzestan 13.8 % (Rafiei et al. 2007), Kerman 7.3 % (Harandi et al. 2011), Golestan 2.34 % (Baharsefat et al. 2007), Zanjan 3 % (Haniloo et al. 2004), Kashan 2.4 % (Arbabi and Hooshyar 2006), Meshkin Shahr 1.79 % (Heidari et al. 2006) and Ilam Province 1.2 % (Aflaki et al. 2005). Various factors contributing the increase in probability of acquiring infection include contact with dog, geophagy and eating raw vegetable (Rokni 2009).

Although some studies mentioned that seropositivity in males were higher than females, our finding is not in agreement with the following studies: Rakhshanpour et al. 2012, Rahimi et al. 2011 and Aflaki et al. 2005. The highest rate of infection was found in 40–60 year old age group with 1.8 % and the least rate was in 20–40 year old age group as 0 %. In Golestan (North of Iran) and Kerman (Southeast) Provinces, the highest rates of the disease were observed in 30–60 and 20–30 year old age groups, respectively (Harandi et al. 2011; Baharsefat et al. 2007). In fact, hydatidosis is considered as a chronic disease of long incubation period (might be 20–30 year) and accordingly a wide range of different ages is noticeable in infected individuals (Torgerson and Deplazes 2009).

Dog is the most important final host which plays prominent role in the epidemiology of cystic echinococcosis. Our results indicate 75 % of infected individuals were dog owner and had a close association with dog. This point is noteworthy to mention that the rate of infection in dog with *E. granulosus* shows a prevalence of 5–49 % in different parts of Iran (Rokni 2009).

In spite of the fact that greengroceries are at high risk of hydatidosis due to their close contact with raw vegetables, our finding indicate that 95 % of examined greengroceries had no knowledge of the disease. This lack of knowledge put them more at risk of infection. Therefore more attention on continues health educational programs of this particular group are required in order to increase their knowledge and awareness about the disease which can be life treating.

As regards residency, in the examined subjects the seropositivity in rural area were more than urban areas. However, urban life showed no significant difference with rural life in some surveys (Rakhshanpour et al. 2012; Heidari et al. 2006); Yang et al. (2008) showed no significant association between CE seropositivity and region.

Interestingly enough during our study on vegetable sellers two significant points were discovered: firstly some of vegetable sellers in Mazandaran and Gilan Provinces were accustomed to eating unwashed raw vegetables as a habit during daily work. However, in the present study no correlation was seen between the seroprevalence rate and

vegetable consumption patterns (washed or unwashed) in infected subjects. Besides, only 3 % of subjects were used gloves during job. Considering all above mentioned points, may put this group more at high risk of acquiring hydatidosis.

In conclusion, the authors deduced that the seroprevalence of hydatidosis in examined greengroceries is relatively high in two studied Provinces. Therefore, more continuous preventive measures should be taken into consideration owing to being a public concern in different Provinces of Iran. Moreover, further serological investigations are needed in other areas on this particular group since the current survey was the first work in Iran.

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Conflict of interest We declare that we have no conflict of interest.

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