

Screening of cystic echinococcosis and toxocariasis in rural inhabitants of Khuzestan Province, southwest Iran

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Abstract. Echinococcosis and toxocariasis are considered two important zoonotic diseases worldwide. The aim of the study was to evaluate the seroprevalence of cystic echinococcosis and toxocariasis and identify the role of variable factors such as age, gender, occupation, level of education, and having contact with domestic dogs and cats in the transmission of the parasites in rural inhabitants of Khuzestan Province, located in the southwest of Iran. A total of 410 rural inhabitants, who had contact with domestic dogs and cats, were randomly enrolled in the current study. Antigen B (AgB) was purified from the hydatid cyst fluid, and the ELISA test was performed for seropositive detection of cystic echinococcosis. *Toxocara* IgG was evaluated by ELISA DRG kit, and the positive samples were examined by Western blotting IgG LDBIO kit to confirm and validate the results of ELISA. Of the 410 sera examined, 20 (4.9%) samples were positive for cystic echinococcosis by ELISA test. A significant association was observed on the different type of occupations and seropositivity ($p < 0.05$), but no significant differences were observed between age groups, gender, and educational levels for cystic echinococcosis. Eight (2.0%) cases were found to be positive for toxocariasis by ELISA, but none of the positive cases was confirmed by the Western blotting test. Our study showed that factors such as education, raising awareness, and health improvement in Khuzestan Province may have influenced the prevalence of echinococcosis and toxocariasis in recent years. Local health and sanitary authorities should pay more attention to the health problems in the rural areas.

INTRODUCTION

Echinococcus, an important parasite of mammals, is the causative agent of echinococcosis (Laurimaa *et al.*, 2015). Cystic and alveolar echinococcosis, diseases of humans and animals, are caused by the larval stages of the genus *Echinococcus*. *Echinococcus granulosus* (*E. granulosus*) and *Echinococcus multilocularis* (*E. multilocularis*) have medical, veterinary, economic, and public health importance. Both *Echinococcus* can infect and cause cystic and alveolar echinococcosis in humans (Okoye *et al.*, 2011, Zhenguan *et al.*, 2008). Iran is one of the

endemic foci of cystic echinococcosis in the Middle East, and annually 1.0% of surgical beds are occupied by these patients. Between 2001–2005, 2083 human cases were reported from Iran (Rokni, 2009). In Khuzestan Province, a prevalence of 13.8% has been reported for human cystic echinococcosis (Rafiei *et al.*, 2007).

Humans might acquire toxocariasis through the ingestion of embryonated eggs via contaminated food, raw vegetables, undercooked meat, geophagia, and or by the ingestion of infected paratenic hosts, such as sheep, cattle, and chicken (Deplazes *et al.*, 2011, Strube *et al.*, 2013). Dogs, as

the final host, play an important role in the transmission of the parasites (Azian *et al.*, 2008). Increasing the number of cats and dogs, particularly stray dogs in villages and the suburbs, may facilitate human infection (Talvik *et al.*, 2006). In European countries, seroprevalence of 2.0% to 44.0% with higher values in rural areas has been reported for human toxocariasis (Deutz *et al.*, 2005, Strube *et al.*, 2013) while in tropical countries, the reported seroprevalence ranges from 63.0% (Chomel *et al.*, 1993) to 93.0% (Magnaval *et al.*, 1994). A seroprevalence study in children aged 6-15 years reported the rate of 2.0% in Ahvaz, the capital of Khuzestan Province (Alavi SM *et al.*, 2011).

Thus, this study was performed to evaluate the seroprevalence of cystic echinococcosis and toxocariasis in rural inhabitants of Khuzestan Province, southwestern Iran, as well as to identify the role of factors such as age, gender, occupation, level of education, and having contact with domestic animals such as dogs and cats in the environmental contamination and the transmission of the pathogens in these areas.

MATERIALS AND METHODS

Ethics statement

This study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (with OG-92141 code).

Study area

Khuzestan Province, one of the 31 provinces of Iran, located in the southwest of the country, extends over an area of 64 746 km² and has an estimated population of 4,531,720. The province consists of plateau regions in the south and west, and mountainous regions in the north and east. The climate of province is dry and semi-dry (Rafiei *et al.*, 2007). The mean annual minimum and maximum temperature is about 9°C and 50°C, respectively. The annual precipitation is 995–1100 mm in the north and 150–256 mm in the south (Zarasvandi *et al.*, 2011).

Sample collection

This cross-sectional study was performed from December 2014 to May 2015 in rural inhabitants of Khuzestan Province (North, Central and South), southwestern Iran. The required information about the aims of the research was provided to all participants. Then they were asked to fill out the questionnaire containing demographic data such as age, gender, occupation, level of education, and having contact with animals. For screening, 4 ml blood specimens were randomly collected from the 410 participants and transferred to the Department of Parasitology, Ahvaz Jundishapur University of Medical Sciences. After centrifugation, sera were kept at -20°C until further examinations.

Antigen B purification for serodetection of cystic echinococcosis

Hydatid cyst fluid collected from slaughtered sheep was aspirated under sterile conditions and was centrifuged at 5000 ×g for 30 min at 4°C in order to remove protoscolex and solid materials. Then 200 ml sheep hydatid cyst fluid was dialyzed against acetate buffer (0.005 M, pH=5) overnight at 4°C. The dialyzed solution was centrifuged at 50000 ×g for 30 min at 4°C. After centrifugation, 20 ml phosphate-buffered saline (PBS, pH=8) was added to the pellet and carefully mixed. The solution was boiled at 100°C in water bath for 15 min and centrifuged at 50000 ×g for 60 min. Eventually, the supernatant was transferred to 2 ml tubes and stored at -20°C (Rogan *et al.*, 1991). The protein concentration was measured by Bradford method (Bradford, 1976).

Enzyme-linked immunosorbent assay (ELISA) for detection of human cystic echinococcosis

ELISA test was performed in 96-well microtiter plates (SPL, Korea). At first, the plates were coated with 100 µl (5 µg/ml) of the AgB in 0.05 M carbonate/bicarbonate buffer (pH=9.6) and incubated overnight (at 4°C). Then, the plates were washed four times with phosphate buffered saline

containing 1.0% Tween-20 (PBS/T, 0.15 M, pH=7.2) and blocked for 2 hrs at room temperature with 300 µl of blocking buffer (PBS containing 1.0% skimmed milk). After a further wash step, a 100 µl aliquot of sera (diluted 1:100 in PBS) was added per well and incubated for 60 min at room temperature. Subsequently, the plates were washed four times with PBS/T. In the next stage, 100 µl of goat anti-human-immunoglobulin G conjugated to alkaline phosphatase (Sigma Aldrich, Saint Louis, USA) was added at a dilution of 1:3000 in PBS and incubated for 60 min at room temperature. After a further wash step, 100 µl (1 mg/ml) per well of a *p*-Nitrophenyl Phosphate, Disodium Salt, Hexahydrate substrate in 0.05 M carbonate/bicarbonate buffer (pH 9.8) was added and incubated for 30 min at room temperature (dark environment). Finally, absorbance values were read at 405 nm by ELISA plate reader (Schweiger *et al.*, 2012). In each run, the positive and negative sera from patients with surgically and pathologically confirmed liver CE and healthy blood donors, were used as positive and negative controls, respectively. The cut-off-values were taken using the mean +2 SD of the negative sera, which were included in all plates.

Enzyme-linked immunosorbent assay (ELISA) for detection of human toxocariasis

ELISA test was conducted using DRG *Toxocara* IgG (EIA-3518, USA) kit according to the manufacturer's instructions. Two drops of the positive and negative controls, included in the kit, were added to the first and second wells. Then 100 µl of each diluted serum samples (1:64) was added to the remaining micro wells containing *Toxocara* excretory/secretory antigen. Following incubation for 10 min at room temperature and washing according to the manufacturer's protocol, two drops of Enzyme Conjugate were added to each well. Incubation and washing were repeated, and then 2 drops of the Chromogen, tetramethylbenzidine (TMB), were added to each well. Finally, the optical density (OD) values were read at 450 nm by ELISA plate reader. OD values of ≥ 0.5 were classified

as positive, and, on the other hand, OD values of ≤ 0.3 were classified as negative.

Western blotting test for detection of anti-*Toxocara* IgG antibodies

To confirm the results of ELISA, the positive samples were analyzed by *Toxocara* Western Blot IgG kit (LDBIO, France) according to the manufacture's protocol. The positive control provided in the kit was included in the experiment.

Statistical analysis

Data analysis was performed using SPSS software, version 21, and Chi-square and Fisher's exact tests. The *P*-value less than 0.05 was considered significant.

RESULTS

Of the 410 participants, 123 (30.0%) were males and 287 (70.0%) were females, respectively. Of these, 20 (4.9%) cases were positive for cystic echinococcosis using ELISA test. Table 1 shows the results of seroepidemiology of cystic echinococcosis according to the gender, age group, educational status, occupation, and keeping dogs at home. Based on the presented data in the table, of the 20 positive samples, 11 (55.0%) and nine (45.0%) positive cases were found in the age range of 21–40 and over 40 years, respectively. No significant differences were observed between different age groups ($p=0.111$). Among the 20 seropositive cases, housewives with a frequency rate of 50.0% (10/20 positive cases) showed the highest percentage, and unemployed participants showed the lowest rate of infection (10.0%). There was a significant association between occupation and infection ($p=0.000$). In addition, Table 1 demonstrates that the frequency of cystic echinococcosis based on gender ($p=0.317$), educational status ($p=0.363$), and having contact with dogs ($p=0.568$) were not significant.

From the 410 examined participants, 8 (2.0%) cases were positive for toxocariasis by ELISA test. No positive cases were confirmed for toxocariasis by the Western

Table 1. The frequency (%) of cystic echinococcosis according to gender, age group, educational status, and keeping dog at home in rural inhabitants of Khuzestan Province, southwest Iran

Category	Group	Hydatidosis				Total	
		Positive		Negative			
		No.	%	No.	%	No.	%
Gender	Male	8/123	6.5	115/123	93.5	123/410	30.0
	Female	12/287	4.2	275/287	95.8	287/410	70.0
Age group	≤ 20	0/36	0.0	36/36	100.0	36/410	8.7
	21-40	11/147	7.5	136/147	92.5	147/410	35.9
	>40	9/227	4.0	218/227	96.0	227/410	55.4
Educational status	Low level literacy	13/307	4.2	294/307	95.8	307/410	74.9
	Intermediate level	3/30	10.0	27/30	90.0	30/410	7.3
	High level	4/73	5.5	69/73	94.5	73/410	17.8
Occupation	Unemployed	2/80	2.5	78/80	97.5	80/410	19.5
	Farmer	5/79	6.3	74/79	93.7	79/410	19.3
	Housewife	10/243	4.1	233/243	95.9	243/410	59.3
	Stockman	3/8	37.5	5/8	62.5	8/410	2.0
Keeping dog at home	Yes	11/200	5.5	189/200	94.5	200/410	48.8
	No	9/210	4.3	201/210	95.7	210/410	51.2

Table 2. The frequency (%) of toxocariasis according to gender, educational status, and keeping cat and dog at home in rural inhabitants of Khuzestan Province, southwest Iran

Category	Group	Toxocariasis				Total	
		Positive		Negative			
		No.	%	No.	%	No.	%
Gender	Male	6/123	4.9	117/123	95.1	123/410	30.0
	Female	2/287	0.7	285/287	99.3	287/410	70.0
Educational status	Low level literacy	5/307	1.6	302/307	98.4	307/410	74.9
	Intermediate level	0/30	0.0	30/30	100.0	30/410	7.3
	High level	3/73	4.1	70/73	95.9	73/410	17.8
Keeping cat at home	Yes	6/175	3.4	169/175	96.6	175/410	42.7
	No	2/235	0.9	233/235	99.1	235/410	57.3
Keeping dog at home	Yes	7/200	3.5	193/200	96.5	200/410	48.8
	No	1/210	0.5	209/210	99.5	210/410	51.2

blotting test. Table 2 indicates the results of seroepidemiology of toxocariasis according to gender, level of education, and keeping dogs and cats at homes among the participants. Of the eight positive samples, 6 (75.0%) and 2 (25.0%) cases were males and females, respectively. A significant difference was observed between males

and females ($p=0.005$), but the correlation between level of education and toxocariasis was not significant ($p=0.281$). The obtained results show that six (75.0%) and seven (87.5%) of the positive cases kept cats and dogs at their homes, respectively. However, no significant differences were observed between toxocariasis and having contact

with cats and dogs ($p=0.078$ for contact with cats and $p=0.033$ for contact with dogs) (Table 2).

DISCUSSION

Although cystic echinococcosis and toxocariasis are two of emerging zoonotic diseases as well as endemic diseases in most parts of Iran, especially in Khuzestan Province, but our knowledge is limited regarding the public health importance and the epidemiology of the diseases in the province (Alavi SM *et al.*, 2011, Rafiei *et al.*, 2007). This research was the first community-based study of the diseases in rural areas of the province. Therefore, the aim of the study was to evaluate the prevalence of cystic echinococcosis and toxocariasis, and to identify the role of different factors including age, gender, occupation, level of education, and having contact with domestic dogs and cats in the environmental contamination and the transmission of the pathogens among rural inhabitants of the province, in southwest of Iran.

The results of this study revealed that of the 410 participants, 20 (4.9%) cases were positive for cystic echinococcosis using ELISA test. The prevalence rate of 4.9% for cystic echinococcosis is consistent with studies performed in different parts of Iran (H.R. Khalkhali *et al.*, 2017, Rafiei *et al.*, 2007). In a systematic review study and meta-analysis, Khalkhali *et al.* evaluated the prevalence rate of cystic echinococcosis in Iran from January 1990 to December 2015, and reported that the weighted prevalence of human cystic echinococcosis was 4.2% (95% CI = 3.0–5.5%). In the review, most cases of human cystic echinococcosis were found in the southern part of Iran, with an outbreak of 5.8% (95% CI = 3.2–9.2%), while the lowest prevalence was in the central parts of Iran, with 2.2% (95% CI = 1.3–3.4%). In addition, the prevalence rates of cystic echinococcosis in northern and western areas were 4.4% (95% CI = 2.3–7.0) and 5.0% (95% CI = 2.7–8.1), respectively. However, more human cystic echinococcosis was observed

in rural areas, mostly among females (H.R. Khalkhali *et al.*, 2017). Most probably, more exposure to sources of infection, such as contaminated soil and close contact with infected dogs, could influence the prevalence of infection among housewives (Rokni, 2009). Furthermore, in 2007 Rafiei *et al.* showed that in some regions of the province, the seroprevalence of cystic echinococcosis was 13.8% by ELISA test (Rafiei *et al.*, 2007). In comparison with Rafiei *et al.*'s study, our study indicated a sharp decrease in the prevalence rate of cystic echinococcosis in Khuzestan Province, which can be due to the improvement of hygiene practices and awareness of majority of people. Despite the fact that with the global warming the possibility of parasites transmission also increases, the observed prevalence was lower than that of previous years (Mas-Coma *et al.*, 2009, Polley and Thompson, 2009).

In addition, in the present study, the prevalence rate of cystic echinococcosis was higher in females than males, but no significant differences were observed between them ($p=0.317$) (Table 1). Similar to our study, in the studies of Khalkhali *et al.* and Rafiei *et al.* the prevalence of human cystic echinococcosis was higher in females, but no significant differences were found between two genders (H.R. Khalkhali *et al.*, 2017; Rafiei *et al.*, 2007). Furthermore, a significant difference was observed between different occupations ($p=0.000$). The highest prevalence of cystic echinococcosis was observed in housewives. This finding is probably due to traditional household tasks of females in endemic areas, such as food preparation, having close contact with domestic dogs, and other activities such as gardening. The greater number of women participating in the study is possibly another effective factor. Although cystic echinococcosis was mostly present in individuals that had contact with dogs (as definitive hosts), no significant differences were observed between cystic echinococcosis and having contact with dogs ($p=0.568$). The results were consistent with Rafiei *et al.*; however, since a prevalence of 64.0% in the central (Sharifi and Zia-Ali, 1996), 20.0% in the northwest (Dalimi *et al.*,

2006), 16.9% in the northeast (Beironvand *et al.*, 2011), 13.2% in the west (Shariatzadeh *et al.*, 2015), and 7.4% in the southeast of Iran have been reported (Eslami *et al.*, 2010), the significant role of domestic dogs in human infection and environmental contaminations cannot be ignored in majority of rural houses, dogs are kept for the purpose of guarding and also as pets. Therefore, infected dogs can play an important role in the transmission of zoonosis to humans through soil contaminated with feces (Eslami and Hosseini, 1998; Zhang *et al.*, 2015).

In this study, higher rate of infection was observed in the age range of 21–40, but no significant differences were obtained between different age groups ($p=0.111$) (Table 1). Similar to our results, Akalin *et al.* indicated that the highest prevalence of cystic echinococcosis was in the age range of 30–39 years (Akalin *et al.*, 2014). Furthermore, the same result has been reported for age groups of 21–40 years in Libya and 21–30 years in Kenya (Eckert, 2001). The association between the level of education, having contact with cats and dogs, and cystic echinococcosis was not significant (Tables 1). In general, it can be concluded that cystic echinococcosis increases with age (Tiaoying *et al.*, 2005). In general, it can be concluded that cystic echinococcosis increases with age (Rafiei *et al.*, 2007; Resaerch, 2008; Zibaei *et al.*, 2013). The intermittent anthelmintic treatment of dogs, monitoring of local slaughterhouses, and controlling dog populations can lead to the lower incidence of cystic echinococcosis, resulting in lower economic burden imposed on the society. Hence, the obtained data from the current study can be helpful in designing more appropriate management programs and safety precautions in cases of human and animal cystic echinococcosis.

Based on the obtained results, of the 410 collected sera, eight (2.0%) cases were positive for toxocariasis by ELISA test (Table 2). No positive cases of toxocariasis were found by western blotting as the confirmatory test. It is worth noting that, most probably, the cause of false positive in ELISA test was cross-reactivity between

toxocariasis and other helminthic infections. The sensitivity of the IgG-ELISA is reported 87.5% by DRG kit. However, in a recent study, the sensitivity and specificity of ELISA test were increased to 92.2 and 86.6%, respectively, when the crude antigens from *T. canis* larvae (TCLA) was used instead of *Toxocara* excretory-secretory antigen (TES) (Jin *et al.*, 2013). One of the important factors in survival of parasite eggs is regional climate. High precipitation, moist soil, and low temperature lead to long-term survival of *Toxocara* eggs in the environment (Macpherson and L., 2013). The prevalence of *Toxocara* eggs was investigated on children playgrounds in Hanover, Germany, during different seasons. The lowest and highest rate were for the months of September and February, respectively (Kleine *et al.*, 2017). Khuzestan Province has dry and hot climate, and in some regions the temperature can reach above 50°C in summer (Mombeni, *et al.*, 2013), so the eggs surveillance can decrease in these areas. This low prevalence was in line with the findings of Nourian *et al.*, study (Nourian *et al.*, 2008) conducted in rural and urban areas of Zanjan (northwest of Iran). In the study, the overall seroprevalence was quite low, that is, 2.7% of 810 children's samples were positive for toxocariasis. Seroprevalence of the parasites was identified in 8/494 (1.6%) children from the urban regions, whereas 4.4% children were positive in the rural areas. In addition, during 2014–2015, Momeni *et al.* evaluated the seroprevalence of toxocariasis in the inhabitants with the age range of 2–20 in northwest of Iran, reporting that 12/397 (3.0%) samples were positive for anti-*Toxocara* IgG. However, the results of the studies were contrary to the findings of Sadjjadi *et al.*'s study conducted in Shiraz, southern Iran, in 2000 (Sadjjadi *et al.*, 2000). In the survey, the overall prevalence was 25.6%. A lower prevalence rate of toxocariasis was reported in the rural (20.2%) areas compared to the urban (30.15%) areas. Moreover, differences in the prevalence can be attributed to the differences in the sensitivity and specificity of the commercial kits as well as the studied groups.

In other countries, Prestes-Carneiro *et al.*'s study (Prestes-Carneiro *et al.*, 2013) carried out in Dona Carmen settlement, Pontal of Paranapanema, São Paulo, Brazil, in 2013 the seroprevalence of toxocariasis was evaluated by ELISA and western blotting tests. They determined some risk factors for people who had antibodies of *Toxocara* species (28 cases out of 194 cases, 14.4%). No significant association was observed between level of education, having contact with cats and dogs, and toxocariasis (Table 2).

CONCLUSIONS

This study provides epidemiological data on the prevalence of cystic echinococcosis and toxocariasis in rural areas of Khuzestan Province, southwestern Iran. The results of this study showed that the prevalence of cystic echinococcosis and toxocariasis was lower than that of previous studies. Probably various factors such as improvement of sanitation, raising awareness, and health education have had an important role in decreasing prevalence of parasitic infections. In spite of this decrease, the findings indicated that cystic echinococcosis is still a serious public health problem in the region. It is suggested that control strategies, including anthelmintic treatment of dogs, controlling stray dogs populations, and education for dog owners, should be implemented to reduce the transmission of zoonotic parasites to the inhabitants of villages.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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