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Original Article

Screening municipal waste collectors for cystic echinococcosis and toxocariasis in southwestern Iran

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Abstract

Introduction: Cystic Echinococcosis (CE) and toxocariasis caused by the larval stages of intestinal dog worms including *Echinococcus granulosus* and *Toxocara* spp. are among the most widespread zoonotic diseases.

Methodology: Four hundred municipal waste collectors were serologically evaluated for CE and toxocariasis. To identify the seropositive cases of CE, an ELISA test was performed using native AgB. *Toxocara* IgG detection was carried out using ELISA DRG kit (USA), and the seropositive cases were then examined by a Western blot kit (LDBIO, France) to confirm the positive ELISA results.

Results: 15 (3.7%) workers were seropositive for CE according to the ELISA. A significant relationship was observed between being seropositive and having contact with soil and dogs. No significant correlations were observed between education and the prevalence of these diseases. Of the 15 seropositive workers for CE, ten worked in district 5 of Ahvaz. *Toxocara* IgG was identified in 11 (2.7%) cases using the ELISA; however, none of them were confirmed by Western blotting.

Conclusion: The 3.7% rate of seroprevalence for CE in asymptomatic municipal waste collectors living in urban regions of Ahvaz suggests a high rate. The higher rate of infection among workers in district 5 is likely associated with the presence of stray and owned dogs in that area of the city. A prolonged exposure to contaminated soil, the lack of awareness about the risk of diseases that can be transmitted through waste and the lack of general availability of suitable personal protective equipment for waste collectors might cause infectious diseases.

Key words: Municipal waste collectors; cystic echinococcosis; toxocariasis; ELISA; Western blotting; Iran.

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Introduction

Echinococcosis is a zoonotic disease caused by the larval stages of tapeworms belonging to the genus *Echinococcus* [1]. Cystic Echinococcosis (CE) and Alveolar Echinococcosis (AE) caused by *Echinococcus granulosus* and *Echinococcus multilocularis*, two main species of the *Echinococcus* genus, have medical and public health importance [1]. In the life cycle of both *Echinococcus* species, domestic and wild carnivores are the final hosts; livestock species are the intermediate hosts of *E. granulosus*; meanwhile, small mammals are the intermediate hosts of *E. multilocularis* and play an important role in the transmission of these cestodes [2]. Human infection occurs accidentally by the ingestion of *Echinococcus* eggs passed into the environment by the feces of infected definitive hosts [3]. Although various organs might be infected by the oncosphere, the liver and lungs are the main organs involved in CE. The liver is involved in 70% of CE cases and the lungs in approximately 20% of the cases. The rest of the organs are infected with CE less frequently [4]. In 40% to 80% of patients, cystic lesions are usually seen as a single cyst. Most CE patients are asymptomatic. As a result, in many cases, patients are incidentally diagnosed by imaging techniques performed for other reasons. In rare cases, the reactions of the immune system can lead to clinical symptoms, such as asthma and anaphylaxis [1].

With the exception of Antarctica, human CE occurs on all continents [5]. An estimated 466 million people living in Central Asia, including Turkmenistan, Tajikistan, Uzbekistan, Kazakhstan, Kyrgyzstan, Mongolia, Iran and Afghanistan, suffer from echinococcosis [6]. In endemic areas, human CE can exceed 50 per 100,000 person-years [1]. The global economic burden of the disease has been estimated up to USD 2 billion in animals [7] and USD 193,529,740 in humans, annually [8].

According to molecular genetic studies, ten genotypes (G1–G10) of *E. granulosus* have been identified [9]. Human CE is mainly caused by *E.*

granulosus sensu stricto, particularly the G1 genotype [2]. Risk factors such as poor education, a pastoral occupation, a history of dog ownership, age, gender and the source of drinking water are associated with human CE [10]. Meanwhile, high precipitation, moist soil and low temperature lead to the long-term survival of taeniid and ascarid eggs and can provide opportunities for human infection; in addition, climate changes affect the survival of *Echinococcus* eggs in the environment [5].

Toxocariasis is another zoonosis caused by the larval stages of *Toxocara canis* and *Toxocara cati,* the adult worm parasite in the small intestine of canine and feline definitive hosts. Infective final hosts can contaminate the environment by excreting eggs in the feces [11]. *Toxocara* spp. eggs are protected from the influence of environmental agents due to their thick shell. Favorable conditions, such as indirect sunlight, appropriate temperature, humidity and oxygen, lead to the survival of the eggs for several years. Nonetheless, exposure to sunlight at 37 °C or quick freezing to -40 °C and consequent heating to 40 °C kill the eggs [12]. As accidental hosts, humans acquire infection by the ingestion of embryonated eggs in contaminated soil and raw vegetables and/ or by the consumption of undercooked meat from paratenic hosts. There is also some evidence that embryonated *T. canis* eggs on the fur of infected dogs could expose humans to the risk of infection [13]. Living in rural areas and close contact with contaminated soil and infected dogs are other relevant factors that may increase the risk of infection [14]. Human toxocariasis manifests itself through clinical symptoms and is classified as neurotoxocariasis (NT), ocular toxocariasis (OT), visceral larva migrans (VLM) and covert toxocariasis (CT) [13]. The global prevalence of human toxocariasis is associated with various factors, such as geographical location, socio-economic status and cultural issues [15].

Public areas such as parks, playgrounds and lake beaches may be contaminated with dog feces. Soil contamination with dog feces and parasite eggs has a potential risk of infection for humans [16]. In urban areas, a large number of dogs and the lack of green spaces for their physiological needs may increase the possibility of soil contamination [17].

Iran is one of the endemic areas of CE in the Middle East [18]. Moreover, several studies have reported *Toxocara* infection among human populations and environmental sources in the center, west and southwest of Iran [19-24]. Khuzestan Province is an endemic area of CE and toxocariasis; however, data on human infections in these regions are limited to a few studies. Another important point is that these studies have focused only on rural communities [25, 26] and children under 15 years old [27]. Since the probability of parasitic infections may be higher among people with lower levels of awareness or those more exposed to transmitting agents, such as soil and solid waste, the present study was designed to investigate CE and toxocariasis among manucipal waste collectors in Ahvaz, southwest Iran. Municipal Solid Waste (MSW), which contains household and commercial waste, might be contaminated with infectious agents such as fungi, bacteria, viruses and parasites [28, 29]. In Iran, collecting solid waste from households, hospitals, factories or streets and green spaces is performed by municipal waste collectors. Moreover, in cities where stray dogs roam the suburban streets, municipal waste collectors work to clean the streets and collect dog feces off the ground.

Manucipal workers, especially those involved in environmental sanitation and cleaning green spaces, who are in contact with soil, might be more at risk of parasitic infections.

Methodology

Ethics Statement

The protocol of this study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (Approval No. IR.AJUMS.REC. 2015-244).

Study area

Khuzestan Province, located in southwestern Iran, near the border with Iraq, is one of the 31 Provinces of Iran. The province covers an area of 63,238 km^2, has an estimated population of 4,531,720, and includes 27 counties. Ahvaz (31° 19′ N 48° 40′ E) is the capital and the largest city of Khuzestan Province that has an estimated population of 1,302,591 [30]. The weather is usually warm and dry in this province, and occasionally humid in the summer [31]. In recent years, dust storms have hit the southwest of Iran. Housing 4% of the country's pollution, Ahvaz is one of the world's most polluted cities [32]. The city is divided into seven municipal districts, and approximately 2419 municipal workers are responsible for collecting solid waste from its grounds.

Sampling

This cross-sectional study was conducted in 2015 using simple random sampling. Of the 2419 workers in Ahvaz County, 400 people between ages 20 to 60 years were randomly enrolled in the study. Before sampling,

the participants were briefed on the study objectives. They were then asked to sign a consent form and complete a questionnaire containing demographic data, including age, gender, education, soil contact and contact with dogs. Approximately 2 mL venous blood sample was taken from each participant. The blood samples were placed in a cold box, labeled with the assigned number, place and date of sampling, and then taken to the Parasitology Department of Ahvaz Jundishapur University of Medical Sciences. After centrifuging, the sera were transferred to 2-mL tubes and stored at -20 °C until they could undergo further examinations.

Antigen B purification

The Hydatid Cyst Fluid (HCF) was collected from the liver cysts of infected sheep slaughtered at Ahvaz abattoir. The HCF obtained was transferred to 50-mL falcon tubes and then centrifuged at 2000×g at 4 °C for 5 min to remove the protoscoleces and solid materials. Then, 100 mL of HCF was dialyzed against 0.005 M acetate buffer (pH = 5) at 4 °C overnight and centrifuged at 50,000×g for 30 min. The supernatant was discarded and 10 mL of phosphate-buffered saline (PBS; pH = 8) was added to the pellet and completely dissolved by vortexing. After boiling at 100 °C for 15 min, it was centrifuged at 50,000×g for 60 minutes. Finally, the supernatant (containing AgB) was transferred to 2-mL tubes and stored at 20 °C to undergo further examinations [33]. The protein concentration was assayed using the Bradford method.

ELISA for the detection of human cystic echinococcosis

The Enzyme-Linked Immunosorbent Assay (ELISA) test was performed to detect seropositive cases of CE. SPL maxi binding plates (SPL, Pocheon, South Korea) with 96 wells were coated with 100 µL (5 μg/mL) of purified AgB diluted in coating buffer (0.1 M carbonate/bicarbonate buffer, pH = 9.6) and incubated at 4 °C overnight. The plates were then washed four times with PBS 0.1% Tween 20 (PBS/T) and blocked with 300 µL of PBS containing 1% Bovine Serum Albumin (BSA; Sigma Aldrich, Saint Louis, USA) for 2 hours at room temperature (RT). After four times washing with PBS/T, 100 µL of sera diluted at a rate of 1:100 in PBS was dispensed to each well and incubated at RT for 1 hour. Afterwards, the plates were washed again as described above, and 100 µL of diluted at 1:3000 in PBS-T anti-human IgG antibody (whole molecules) conjugated to alkaline phosphatase produced in goat (Sigma Aldrich, Cat No. A1543, Saint Louis, USA) was added to each well and incubated for 1 hour. After five times washing, 100 µL/well (1 mg/mL) of *p*-Nitrophenyl Phosphate, Disodium salt and Hexahydrate substrate (Sigma Aldrich, Cat No. 71768, Saint Louis, USA) in 0.05 M of carbonate/bicarbonate buffer (pH 9.8) was added and kept in a dark environment at RT for 20 min, and the absorbance was measured at 405 nm using an ELISA plate reader [26]. In each run, the positive sera from the patients with surgically- and pathologically-confirmed liver CE in Ahvaz hospitals and the negative sera from healthy blood donors were used as the positive and negative controls, respectively. The cut-off points were taken using the mean +2 SD of the negative sera, and were included in all the plates.

ELISA for the detection of human toxocariasis

According to the manufacturer's instructions, all the sera were examined using the DRG *Toxocara* IgG (EIA-3518, Mountainside, NJ, USA) kit for the detection of toxocariasis. Finally, the absorbance values were measured at 450 nm by an ELISA plate reader.

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

To confirm the results of the patients who were deemed positive by the ELISA test, a *Toxocara* Western Blot IgG kit (LDBIO, Lyon, France) was used according to the manufacture's protocol.

Statistical Analysis

The data were analyzed in IBM SPSS Statistics version 16 (SPSS Inc., Chicago, IL, USA) using the Chi-square test. *P*-values less than 0.05 were considered statistically significant.

Results

Overall, 400 male municipal waste collectors (since, in Iran, municipal workers are only male) with a mean age of 35.6 years and an age range of 20-60 years were included in the present study. A total of 84 (21.0%) of them worked in district 1, 44 (11.0%) in district 2, 46 (11.5%) in district 3, 65 (16.2%) in district 4, 58 (14.5%) in district 5, 54 (13.5%) in district 6 and 49 (12.3%) in district 7 of Ahvaz.

The participating workers were divided into groups by age range, including a 20-30-year-old group (n = 121, 30.2%), a 31-40 group (n = 175, 43.8%), a 41-50 group (n = 88, 22.0%) and a 51-60 group (n = 16, 4.0%). Most of the participants were from the age group of 31 to 40 years (43.8%). As for education, 60 (15.0%) of the participants had a high school diploma and 340 (85.0%) were high school dropouts. Among examined

Table 1. Seroprevalence of cystic echinococcosis and toxocariasis (%) in Ahvaz municipal waste workers by IgG ELISA based on age, educational level, residence area, and contact with soil and dog.

Characteristic		Cystic echinococcosis			Toxocariasis		
		Positive No (%)	Negative No (%)	*P*-value	Positive No (%)	Negative No (%)	*P*-value
Age groups	20-30	1 (0.8)	120 (99.2)	0.169	4 (3.3)	114 (94.2)	0.300
	31-40	10 (5.7)	165 (94.3)		6 (3.4)	169 (96.6)	
	41-50	3 (3.4)	85 (96.6)		0 (0.0)	88 (100.0)	
	51-60	1 (6.3)	15 (93.8)		1 (6.3)	15 (93.7)	
Educational level	No education	3 (7.7)	36 (92.3)	0.253	2 (5.1)	37 (94.9)	0.456
	Middle school	8 (4.1)	188 (95.9)		4 (2.0)	192 (98.0)	
	High school	4 (3.8)	101 (96.2)		2 (1.9)	103 (98.1)	
	Diploma	0 (0.0)	60 (100.0)		3 (5.0)	57 (95.0)	
Residence area	Urban	1 (0.5)	206 (99.5)	0.000	0 (0.0)	207 (100.0)	0.000
	Suburban	14 (7.3)	179 (92.7)		11 (5.7)	182 (94.3)	
Soil contact	Yes	15 (3.8)	375 (96.2)	0.527	11 (2.8)	379 (97.2)	0.590
	No	0 (0.0)	10 (100.0)		0 (0.0)	10 (100.0)	
Contact with dog	Yes	12 (13.5)	77 (86.5)	0.000	9 (10.1)	80 (89.9)	0.000
	No	3 (1.0)	308 (99.0)		2 (0.6)	309 (99.4)	

workers, 15 (3.7%) workers proved seropositive for cystic echinococcosis using the ELISA. All the positive cases were asymptomatic. As for age group, ten (66.6%) workers were in the 31-40 age group, three (20.0%) in the 41-50 age group, and one each (6.7%) in the 20-30 and above-50 age groups. Although all the seropositive workers were high school dropouts, no significant relationships were observed between education and CE ($P = 0.253$). Concerning soil and dog contact, all the seropositive workers had a history of close contact with dogs, and due to their occupation, they were in contact with soil as well. In two (13.3%) of the workers who were seropositive for CE, the ELISA findings were also positive for toxocariasis (Table 1). The highest frequency, i.e. 10 (66.6%), belonged to the workers involved in collecting waste in district 5 (Table 2).

Out of the 400 examined workers, 11 (2.7%) were positive for anti *Toxocara* IgG according to the ELISA test. None of the ELISA-positive cases were confirmed by Western blotting. The association between age group, education, soil contact and positive serology was thus not significant ($P > 0.05$). Nonetheless, the results showed a significant correlation between positive serology and place of residence and contact with dogs ($P < 0.05$; Table 1). The highest percentages of positive cases belonged to district 1 (n = 4, 4.8%) and district 5 (n = 3, 5.2%), respectively (Table 2). In two workers who were seropositive for CE, the ELISA test was positive for toxocariasis as well.

Discussion

Dogs can play different roles, such as guarding, hunting and acting as shepherds and pets in human societies [1,34]. Alongside these benefits, dogs may also be the reservoirs, transmitters [35] and possible sources of zoonoses such as echinococcosis and toxocariasis [36]. In both diseases, human infections occur accidentally through the oral ingestion of embryonated eggs [16]. One of the main transmission routes of zoonoses, however, is having direct contact with contaminated soil [37]. Some zoonotic helminths

Table 2. Seroprevalence of cystic echinococcosis and toxocariasis (%) in Ahvaz municipal waste workers by IgG ELISA based on districts of the city.

District	Cystic echinococcosis			Toxocariasis		
	Positive No (%)	Negative No (%)	*P*-value	Positive No (%)	Negative No (%)	*P*-value
1	2 (2.4)	82 (97.6)	0.000	4 (4.8)	80 (95.2)	0.244
2	1 (2.3)	43 (97.7)		0 (0.0)	44 (100.0)	
3	0 (0.0.)	46 (100.0)		2 (4.3)	44 (95.7)	
4	0 (0.0)	65 (100.0)		0 (0.0)	65 (100.0)	
5	10 (17.2)	48 (82.8)		3 (5.2)	55 (94.8)	
6	1 (2.0)	53 (98.0)		0 (0.0)	54 (100.0)	
7	1 (2.0)	48 (98.0)		2 (4.1)	47 (95.9)	
Total	**15 (3.7)**	**385 (96.3)**		**11 (2.7)**	**389 (97.3)**	

can be transmitted by contaminated soil, such as *Toxocara* spp. [16]. More than 1.5 billion people are estimated to be infected with soil-transmitted helminths globally [38]. Due to their low awareness and direct contact with soil, farmers and waste collectors may be at a higher risk of soil-transmitted helminthic infections. Evaluating parasitic infections, including echinococcosis and toxocariasis, in high-risk groups in endemic areas is therefore considered essential.

In Iran, municipal waste collectors must wear gloves, facemasks and boots at work. The researchers' personal observations, however, showed a general lack of personal protective equipment for these workers. In addition, due to their poor awareness and economic status, regular checkups may be neglected by this group.

In the present study, 15 (3.7%) of the municipal waste collectors proved positive for CE as per the ELISA test. Ten (66.6%) of the positive cases worked in district 5. In district 5, which is located on the outskirts of the city, some people keep livestock as well as guard dogs for their house. Stray dogs may also roam the streets in this district. In a recent seroepidemiological study of the rural inhabitants of Khuzestan Province who kept dogs at their home, the prevalence of CE was reported as 4.9% [25]. In another seroepidemiological study of Khuzestan Province, CE was reported in 1.0% of the normal rural population [39]. According to a study by Rafiei *et al*. in 2007, the seroprevalence of CE was 13.8% in the nomads of some regions of the province [26]. Although all previous studies were performed on rural inhabitants, the highest CE infection was observed in the present study among the waste collectors working in district 5; this group was found to be at a higher risk of exposure to dog feces. In all three noted studies, the rate of infection was higher among women than men [25,26,39]. In the present study, all the participants were male; therefore, no possible association could be found between the disease and gender. In a review study in Iran, CE was reported to be more prevalent among women than men [40]. There is also a possibility that the higher exposure of women to infective agents is the reason for the greater prevalence of infection among them. The workers in the age group of 31-40 years showed the highest prevalence of infection, i.e. 20.0%. It seems that most cases of CE in Iran occur in the age range of 20-40 years [40]. Although these findings are consistent with the results of a study in Turkey [41], the 3.7% prevalence compared to the noted study on rural inhabitants was noticeable, since the population investigated in the present study also included urban inhabitants [25]. Since 14 (93.3%) of the infected workers lived in the suburbs of Ahvaz, the relationship between CE and place of residence was statistically significant ($P < 0.05$). Stray dogs roam the streets of the suburbs of Ahvaz, and cleaning the streets and collecting dog feces from the ground is performed by municipal waste collectors. Since *E. granulosus* eggs can survive for several months in regions with a warm climate, CE infection can be acquired by handling egg-containing feces or egg-contaminated soil [2] in municipal waste collectors without adequate protective clothing. The collection of dog feces by waste workers with inadequate awareness concerning zoonotic diseases might therefore be a serious health threat.

Toxocariasis, another soil-transmitted helminthic disease, was assessed in municipal waste collectors. Based on the ELISA findings, 11 (2.7%) of the examined workers were positive for anti *Toxocara* IgG. None of the positive cases, however, were confirmed by Western blotting. In two workers, the ELISA test was positive for both CE and toxocariasis. Since other helminthic infections may be responsible for the false positivity of the ELISA for toxocariasis, any ELISA positive samples should be subsequently tested by Western blotting [42]. According to the LDBIO kit instructions, the specificity of the 24-35 bands from the E/S antigen is 100% and their sensitivity is often significantly higher than that of the E/S ELISA screening tests. The prevalence of 2.7% reported by the ELISA test is probably related to other helminthic infections. In a recent study conducted on the rural inhabitants of Khuzestan Province, toxocariasis was not observed in any of the examined participants [25]. Along with the trend of global warming, Khuzestan climate has also changed in recent years, and the temperature can exceed 50 °C at times in some regions [43]. The increasing temperature might be the main cause of the reduction of human toxocariasis in Khuzestan Province in recent years. This decline could also be explained by the age groups studied. In a study by Alavi *et al*. (2011), the seroprevalence of toxocariasis was reported as 2.0% among hypereosinophilic children under 15 years old in Ahvaz. These results were reported only based on an ELISA test, and the positive cases were not examined by Western blotting [27]. In Iran, there is no data on toxocariasis in municipal waste collectors; however, several studies in the northwest, south and west of Iran have reported seroprevalences of 2.7%, 25.6% and 22% for toxocariasis in children [44-46]. Contrary to the present study, all previous studies were performed on children and only using the ELISA test. In a study by

Alvarado-Esquivel in Mexico, 13.0% of waste collectors proved seropositive for toxocariasis [47]. The most important difference appears to be the climatic conditions. Temperature is one of the most important factors involved in *Toxocara* egg embryonation [48]. In a study by Masoudi and Elhaeesahar, climate data were collected from 17 weather stations in the province from 1951 to 2012. The results showed that 88.31% of the province had become warmer [49]. Ahvaz, the capital of the province, with a temperature above 50°C in the summer, is one of the hottest cities in the world [50] and it can therefore be argued that, in addition to the improved awareness and health status of the people in this province, climate change has also been effective in reducing parasitic infections such as *Toxocara* in the region.

Conclusion

From an epidemiological point of view, a seroprevalence of 3.7% for CE observed among municipal waste collectors is noteworthy. Considering that all the seropositive cases were asymptomatic, periodic medical examinations are recommended in high-risk groups. Given that municipal waste collectors are involved in the collection and disposal of toxic, hospital, infectious, inflammable and other types of wastes, increasing the workers' awareness about these issues is critical for the prevention of infection via contaminated soil or waste. Another noteworthy point is that ten of the CE-positive workers worked in district 5. In this district of Ahvaz, some people tend to keep dogs to guard their house and there is a larger presence of stray dogs in this area, which are factors that should not be overlooked. The present findings offer new information to the local authorities for implementing educational programs to increase the awareness of municipal waste collectors and improve their health statues. The municipal authorities should also devise programs to control the presence of stray dogs in the outskirts of Ahvaz.

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Authors' contributions

MB conceived and designed the experiments; RD performed the experiments; MB analyzed data; RD, AR, MT and MB contributed reagents/materials/analysis tools; MB, AR, and MT wrote the paper.

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