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Original papers

Prevalence of *Toxoplasma* and *Echinococcus* IgG antibodies in slaughterhouse workers, a serosurvey in Northeast Iran

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ABSTRACT. Cystic echinococcosis and toxoplasmosis are two human parasitic diseases. Butchers and slaughterhouse workers are in close contact with body fluids and tissues of ruminants. To investigate the prevalence of *Toxoplasma* and *Echinococcus* IgG antibodies in slaughterhouse workers in Northeast of Iran, Mashhad, 2016. This cross-sectional study was performed on all personnel working at the largest industrial slaughterhouse of Khorasan Province. Serum samples were taken and kept frozen until used. IgG against *Echinococcus* and *Toxoplasma* were quantified using commercial ELISA kits. A questionnaire addressing possible risk factors of infection acquisition was filled by participants. Out of 91 male participants, 58.2% were positive for toxoplasmosis, 5.5% were positive for cystic echinococcosis and 3.3% were positive for both. Except using gloves, and gown and boots, other personal protective equipment (PPE) were not completely used by all personnel; mask 38%, tool disinfectants: 12%, face and hand disinfection: 14.3%. There was a high risk of *Toxoplasma* in slaughterhouse workers, however, such finding was not observed in *Echinococcus* parasite. Importance of PPE and tool disinfectants to reduce the risk of zoonotic infections in the workplace should be emphasised.

Key words: *Toxoplasma*, *Echinococcus*, IgG antibodies, slaughterhouse, Iran

Introduction

Toxoplasma gondii is a protozoan parasite that infects a vast range of warm-blooded animals as well as humans. Three stages of this protozoan naturally exist; tachyzoites, bradyzoites and oocysts. Tachyzoites (active form) and bradyzoites (an inactive form of *Toxoplasma* cyst) take part in the asexual cycle in humans and other intermediate hosts. The third form of the organism, oocysts, are excreted through faeces of cat who is definitive host [1]. Ingestion of oocysts via contaminated soil, water or vegetables or meat cysts can infect humans [2,3]. The infection may be transmitted transplacentally from mother to fetus during pregnancy [4]. High environmental survival of oocysts for even more than one year is well documented, this capacity plays an important role in disease transmission [5,6]. Seroepidemiology varies in different parts of the world and factors such as climate, meat-eating habits and level of hygiene affect it. Occupations involving close contact with raw meat might be considered as potentially at risk of seropositivity [7].

In addition, echinococcosis, another parasitic disease, is considered as an occupational health problem for sheep farmers, dog owners and shepherds in endemic areas and slaughterhouses are considered as a “hotspot” of the infection [8,9]. Hydatidosis is a major public health problem in many countries that is caused by *Echinococcus granulosus* [10–13]. This tapeworm lives in the

small intestine of domestic and wild canids. Dogs spread large numbers of parasite eggs on the agricultural fields which directly infect intermediate hosts such as sheep and cattle as well as humans. Human behavior in contact with dogs plays an important role in the transmission of infection. Noteworthy, eggs might be present in the contaminated wool of animals. When eggs are ingested by humans, the oncospheres are liberated from the eggs and migrate via the bloodstream to the liver, lungs, and other tissues and organs to develop into hydatid cysts [7]. As butchers and slaughterhouse workers are in close contact with body fluids and tissues of ruminants, may be at a higher risk of infection acquisition. This study investigated the prevalence of *Toxoplasma* and *Echinococcus* IgG antibodies in slaughterhouse workers in Mashhad, the second most populous city in Iran and the capital of Razavi Khorasan Province located in the northeast of the country.

Materials and Methods

This cross-sectional study was performed on all personnel working at the largest industrial slaughterhouse of Khorasan Province, Mashhad, 2016, after acceptation to participate in the study. Each participant filled a checklist containing demographic information, occupation, kind of ruminants dealing with, history of hand injury and using personal protective equipment (PPE) including mask, gown, glove, and boot. The study was performed with respect to ethical principles stated in the Declaration of Helsinki. This study was reviewed and approved by the ethical committee of Mashhad University of Medical Sciences (MUMS-940438).

Serum samples were kept at -80°C until experimental assay. IgG antibodies against *Toxoplasma* and *Echinococcus* were measured using the ELISA technique by using anti-*Toxoplasma* IgG and anti-*Echinococcus* IgG kits from Pishtazteb co. Tehran, Iran. Both kits applied indirect ELISA systems. The assay was performed based on manufacturers' instructions, briefly, serum samples were added to antigen-coated wells. After the definite incubation time, HRP conjugated anti-human IgG antibody was added. In each step, plate was washed to remove excess unbound material. Finally, the chromogen substrate was added to develop color change. Next OD values were read at 450 and 630 nm in a microplate spectrophotometer. Negative and positive controls were provided by the kit. Cutoff values and index to determine negative and positive results were calculated based on OD values according to the kit protocol. The sensitivity and specificity of these kits were reported to be 91% and 96% for *Echinococcus* and up to 100% for *Toxoplasma* according to the kit manufacturer.

Descriptive analysis (frequency, mean and standard deviation, median, interquartile range) and inferential analysis (Chi-squared test) were performed using SPSS software version 11.5. All tests were two-tailed and the significance level was considered as lower than 0.05.

Results

Ninety-one male workers were included with a mean(sd) age of 38.7(8) years. Median of work

Table 1. The frequency of using personal protective equipment in slaughter house workers of Mashhad, Iran

		Frequency	Percent
Using mask	Always	35	38.5
	Sometimes/rarely	56	61.5
Using gloves	Always	86	96.6
	Sometimes/rarely	3	3.4
Using gowns	Always	87	95.6
	Sometimes/rarely	4	4.4
Using boots	Always	87	95.6
	Sometimes/rarely	4	4.4
Tool disinfection	Always	11	12.1
	Sometimes/rarely	80	87.9
Face-hand disinfection	Always	13	14.3
	Sometimes/rarely	78	85.7

Table 2. Regression analysis for prediction of seropositive *Toxoplasma* and *Echinoccocus*

	Toxoplasmosis		CE	
	Odd Ratio	P-value	Odd Ratio	P-value
Age	1.1	0.02*	1.4	0.36
Work Duration (year)	1.3	0.03*	1.13	0.99
Job (butcher)	2.16	0.21	–	–
Animal visceral contact(≥1per week)	0.53	0.46	0.30	0.33
Using mask	1.30	0.54	–	–
Using gloves	0.34	0.37	–	–
Using gowns	1.41	0.55	–	–
Using boots	1.41	0.55	–	–

duration in the industrial slaughterhouse was 16 years with an interquartile range of 8 to 19 years. Fifty-three persons (58.2%) were positive for toxoplasmosis, also 5 persons (5.5%) were positive for cystic echinococcosis, 3 workers (3.3%) were positive and 36 persons (39.6%) were negative for both infections. There was no significant relation between toxoplasmosis and cystic echinococcosis positivity (p=0.65).

Persons who were positive for toxoplasmosis and cystic echinococcosis had mean(sd) age of 35.6(4) years and mean(sd) duration of work was 14.3(3.7) years, all of them rarely used tool disinfection as well as face-hand disinfection protocol. Moreover, nobody used a proper mask.

The most prevalent jobs in toxoplasmosis positive cases were sheep butcher (49%, 26 persons), cow butcher (42%, 22 persons) and official (9%, 5 persons) (p=0.40). All five positive cases for cystic echinococcosis were sheep butchers (100%). Positive individuals for toxoplasmosis were older (5.6 years) than negative ones (41.0±7.7 versus 35.4±7.3 years old) (p<0.001). Also, the duration of work history was 4.8 years longer in toxoplasmosis positive individuals (15.5±6.5 years) compared to negative ones (10.7±6.6 years) (p=0.002). Regarding the application of PPE, 97% of slaughterhouse workers always used gloves, and in the second rank, gowns and boot (both 95.6%) (Table 1).

Logistic regression was used for determining important risk factors for toxoplasmosis and cystic echinococcosis using the Enter method for modelling of the relationship between disease and various participants' variables (Table 2). Age, butcher job, dealing with animal visceral more than once a week, and using PPE were entered in regression analysis. The result of regression showed that with increasing age (OR=1.1, p=0.02) and work duration (OR=1.3, p=0.03) odds of toxoplasmosis were increased in workers, although age and work duration was correlated and one of them selected for final analysis. Other variables such as using PPE did not show a significant effect.

Discussion

This survey showed a high *Toxoplasma* seroprevalence among slaughterhouse personnel. Mansouri et al. [14] reported a rather high seroprevalence of toxoplasmosis in Iran as 37.6% in women and 33.3% in men. Also, a seropositivity of 34.4% (95%CI=24.9-43.9) has been proposed in blood donors [14]. In Mashhad, our findings showed a higher *Toxoplasma* seropositivity in slaughterhouse workers than the normal population which was reported to be around 31% [15].

According to previous studies, it is possible that women might have higher exposure due to handling raw vegetables contaminated with oocysts produced by a cat [16]. Considering that all abattoir personnel were male, the high seropositivity among these workers is expected due to occupational exposure.

Different factors contribute to the seroprevalence of toxoplasmosis in Iran like cat density. Iran is generally located in a moderate to warm climate suitable for oocytes survival and is well known for a high density of stray and domesticated cats.

We observed a weak correlation between *Toxoplasma* seropositivity and age, which probably is due to the high infection rate, as it has been reported in some previous studies [17,18]. Although several studies have indicated an increase in seroprevalence with age [16,19]. From an

epidemiological perspective, this contradictory finding may be due to differences in the prevalence and variety of transmission patterns.

The overall prevalence rate of toxoplasmosis in livestock of Iran has been estimated as 31% (95%CI =0.25-0.35) in sheep, 27% (95%CI=0.14-0.42) in goats, and 18.1% (95%CI=9.9-28.2) in cattle, respectively [20,21].

The relative importance of the risk factors varies between countries due to differences in cultural patterns and climatic factors affecting oocyst survival; for instance, in most studies, rural residency has been shown to be a higher risk for *Toxoplasma* [18].

Working with raw meat in hyperendemic areas seems to be a neglected potential risk of infection acquisition. Slaughterhouse workers are occupationally exposed to *Toxoplasma*. Contamination of knife with chopped meat, droplet or plasma, environmental contamination, as well as scratched hand or open wounds, contact with raw meat, might transmit parasites to humans. Tissue cysts commonly are formed in skeletal muscle and may remain lifespan of the host. Accidental inoculation of the pathogen may occur in slaughterhouses.

Higher risk for toxoplasmosis in workers of slaughterhouses has been also previously reported and even an increase in seropositivity along with the duration of employment and age [22,23]. Though other studies did not report such a correlation [24].

On the other hand, we did not observe high seropositivity against cystic echinococcosis (CE) among all workers. The prevalence rate of CE based on hospital cases is different in Iran with a rate of >1% of total population (12) and this is the most commonly used index of CE [25,26].

The range of CE in Iran has been shown to ranging from 0.2% in Tehran (the capital) to 15.4% in Khorramabad (west of Iran) [27]. The annual surgical cases of CE in Iran are reported to be 1–2 per 100,000 populations and the seroprevalence rate of CE is 3–19% in different areas of the country [28].

A wide range of infection rate in ultimate and intermediate animal hosts has been reported in Iran. Studies have reported 2% to 63% of dogs and 1.5% to 70% of herbivorous animals such as sheep, goats and camels to be infected by *Echinococcus* [29–34]. This wide range might be due to the climate and cultural differences among different socio-geographic distinction of Iran. Still, no information is available about the intermediate host's seropositivity in Northeastern Iran and due to high variance, a definite assumption is not possible [27,35]. Though as discussed by Shafiei et al., due to lower livestock farming, and consequently lower intermediate hosts for this helminths, the infection rate should not be in the upper range [27]. Also, the existence of industrial slaughterhouse is another important factor because the infected viscera of intermediate hosts are safely removed from the abattoir and are not consumed by the final host. On the other hand, on house/non-industrial slaughtering the infected viscera might be consumed by dogs and the life cycle of the parasite is completed. Though one should consider that even with a low infection rate in livestock, slaughterhouse personnel are daily exposed to a massive number of livestock and a summative exposure rate is noticeable.

It seems logical that home slaughtering of livestock might be a potential risk for acquisition of the pathogen especially in an area that the infection is endemic in the intermediate host [36]. However, we did not find higher anti-*Echinococcus* antibody in slaughterhouse workers. Similar results have been previously reported [37]. It should be noted that brain, lung and other high-risk organs like liver are not generally butchered in the industrial slaughterhouse compared with the non-industrial slaughtering of livestock. Though this finding does not rule out the occupational risk for CE. A positive relationship between some other jobs such as farm work and CE has been observed [7].

Different factors contribute to CE. The prevalence of CE infection is usually higher in rural inhabitants rather than in urban ones mainly due to close contact with un-vaccinated dogs, farming activities and lower hygiene [26,38,39]; also most of the studies have shown that CE is more common in females than males [27,38,40], though some studies did not report such gender predominance [41]. It seems that daily handling and washing raw vegetables contaminated with *Echinococcus* eggs might be the main reason for the higher infection rate in females. In addition to this, housewives in the rural area generally are involved with farming as well as sheep and cattle raising activities. Most slaughterhouse workers are males and they are not affected by additional ways that generally women are at risk.

None of our serologically *Echinococcus* positive workers was aware of their infection. It is known that the majority of cysts will remain asymptomatic and even spontaneously degenerate in some individuals; and all progressive, stable or even

degenerated cysts remain reactive in the serologic analysis.

Shepherds are probably at risk not only for handling sheep but also in close contact with cattle and dogs. These dogs are usually in far rural areas in where, unlike modern cities, vaccination and pet clinics are not available.

Also, it has been proposed that close contacts of dogs and sheep in a rural area might contaminate the sheep wool and then later transfer the eggs to the farmers [42]. However, such wool contamination does not seem to be the main problem in slaughterhouses, because eating and drinking are not allowed during working in industrial slaughterhouses and personnel must change clothes and wash hands before eating and drinking in the tea room and dining places based on current rules.

This survey found a higher risk of *Toxoplasma* in slaughterhouse workers, though such job-related risk was not observed in the other parasite, *Echinococcus*. This finding gives a new view on how these pathogens are transmitted and increases understanding about the occupational hazard of these zoonotic infections in a place with close contact to livestock like an industrial slaughterhouse. It should be noted that such *Echinococcus* serological tests may give false-positive results, due to cross-reactions with other helminthic diseases, such as taeniosis, fasciolosis and toxocarosis [43] or non-infectious conditions, such as cancer, pregnancy and autoimmune diseases [39]. Similarly, in the case of *Toxoplasma* most currently available serologic tests for *T. gondii* may show some level of cross-reactivity with related coccidia, in particular with *H. hammondi* and *N. caninum* [44]. So, the presence of a specific antibody alone does not confirm the diagnosis, as people may be seropositive for a number of reasons, such as previous exposure to the parasite or cross-reactivity with other conditions. However, due to high specificity and sensitivity of both kits, it seems reasonable that possible minor false positive or false negative results of our study are negligible.

Large-scale epidemiological studies should be carried out to establish the health impacts on workers and later society and to set instructions and guidelines to improve PPE. The PPE tools quality and proper usage of these equipment should be regularly monitored. This can lead to a reduction of the adverse outcomes of these zoonotic infections.

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