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Prevalence and related potential risk factors of *Toxoplasma gondii* infection among slaughterhouse workers and animals in Makkah

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ABSTRACT

Toxoplasma gondii is a protozoan parasite that causes a parasitic disease known as toxoplasmosis. The disease transmits from animals to humans via the eating raw or insufficient cook meat or by contact with raw meat. The main aim of the present study was to define the prevalence and the associated risk factors of the disease in workers and animals in slaughterhouses in Makkah.

The study was based on a cross-sectional study in humans and animals including, sheep, goats, cattle, and camels. Data were collected using a short questionnaire. Approximately 108 blood samples were collected from workers included the butchers and the workers and 160 blood samples of the slaughterhouse animals. Serum samples were kept in -80° C till examined by using ELISA IgM and IgG tests. The data analyzed by using SPSS version 25.

The result showed that the prevalence in workers was 0.9% and 25% using IgM and IgG, respectively. In animals, all the results were negative using ELISA IgM and IgG tests. The result showed that there was a correlation between chronic infection and contact with a cat ($p<0.015$).

The study focussed on the *T. gondii* in slaughterhouse workers which found that both acute and chronic toxoplasmosis. The results also showed no infection was registered in different species of animals in both slaughterhouses.

1. Introduction

Toxoplasma gondii (*T. gondii*) is a protozoan parasite that causes a parasitic disease known as toxoplasmosis. The causative agent of toxoplasmosis, the parasite called *T. gondii*, is a sporozoan belongs to coccidian. Infection caused by this parasite is intricately secured to its complex life cycle. Several mammals are hosts to infection and act as intermediate hosts, although domestic cats and other felines are the only definitive host [1]. Toxoplasmosis is not transmitted from person-to-person, except congenitally or vertically from mother-to-child, so that a woman who is recently infected with *T. gondii* can pass the infection to her unborn child through the placenta or during a blood transfusion or organ transplantation [2-3]. Horizontally, the intermediate hosts in nature can be infected by ingesting oocysts in water, plant material, or soil [4]. Humans can get the infection by different routes, including cutting meat or eating undercooked meat of animals harbouring bradyzoite [5]. The prevalence of infection by *T. gondii* in a different population of animals is varied. It was found that the parasite infected a varied range of animals existing in different environments [6]. Similar to most protozoa causing disease, the prevalence of toxoplasmosis is higher in developing areas. However, the parasite *T. gondii* is one of the protozoa parasites that have maintained a significant prevalence in developed countries such as the USA [4]. This high level of prevalence in humans may be due to the fact that people can get the infection with *T. gondii* by several routes. Subsequently, the level of heat used in cooking interrupts bradyzoites, but infections can occur via ingesting undercooked meat. Also, another factor of concern is that animals used in the food chain differ in their bradyzoite resistance and, therefore, their capability to infect people could be different. The current reason gives an answer to the

question of why the consumption of meat from some species, such as bovines, which are relatively resistant to *T. gondii*, is a less common source of transmission as compared to the ingestion of meat from other animal food sources such as ovine or porcine species [7]. While the effects of fetal infection can be devastating, active fetal infection is a relatively rare event in addition to the fact that *T. gondii* prevalence increased with age [8, 9]. Occupational risks related to augmented *T. gondii* contact, including working with animals and gardening were studied [10]. In Saudi Arabia, there were numerous studies done to estimate seroprevalence, and all studies were conducted among pregnant women. Immunosorbent Assay (ELISA) was used to determine the prevalence rate among pregnant women in Jazan and found that the overall prevalence was 24.1 % [11]. In Najran, hemagglutination was used and it was estimated that 20.8 % were positive [12]. In Riyadh, the seroprevalence of hemagglutination test indicated that 38% of pregnant women were positive to *T. gondii* [13]. Another study done in south western Saudi Arabia among pregnant women used Enzyme Linked Immunosorbent Assay (ELISA) and found that about 38 % of women were positive to *T. gondii* [14]. In the Eastern region, the estimated seropositive test of *T. gondii* gave a prevalence of 25 % by using ELISA IgG and 5% by using ELISA IgM [15]. In Dhahran they found that the overall prevalence of *T. gondii* was 28.5% by using ELISA IgG and 3 % by detection IgM [16]. In the Aseer region, they used a polymerase chain reaction (PCR) and found that 41% was positive to *T. gondii* [17]. The study aimed to determine the prevalence of the disease in workers and animals in slaughterhouses in Makkah.

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2. Material and Methods

2.1. Study area

The study was located in Makkah lying in the west of Saudi Arabia, north west of Taif, east of Jeddah and south of Madinah. Makkah is location of the Kaaba which is visited annually by more than 20 million visitors, including pilgrims and tourists. The total population of the Makkah region is 8,557,766, of whom 4,516,577 are Saudi nationals, and 4,041,189 are non-Saudi residents, representing 47% of the total number of residents of the region.

2.2. Study population

Approximately 43 (39.8%) of the study population were butchers, and 65 (60.2%) were workers. The criteria of selection was included all people working inside the slaughterhouse and in real contact with meat during the time of the study. The criteria excluded the employees and the housekeepers. Their age ranged between 22 - 61 years with a mean of 40.49 years. Samples were collected from Al Ka'akya 97 (89.8%) and Al_Muasim 11 (10.2%). Their duration of working in slaughterhouses ranged between 1 to 45 years with a mean of 14.72 years. The distribution among nationality was found to be 92 (85.2%) from Bangladesh, 9 (8.3%) from Egypt, 4(3.7%) Pakistan and 3 (2.8%) were African.

2.3. Study design

A cross-sectional based study was done in people working in meat processing butchers or workers holding, cutting, and disposing of waste. The study was conducted during the period between March and April 2019.

2.4. Sample size

Data and blood samples were collected from 109 human workers who agreed to participate in the study. Also, 160 samples from different kinds of animals were collected from the slaughterhouse in Al_Kaa'kyah and Al_Muaysim in the Makkah area.

2.5. Data collection

Consent Forms: Approval was obtained from the Ethical Committee in the Faculty of Public Health and Health Informatics, Umm Al Qura University. The purpose and procedures of this study were explained to all participants, and written informed consent was obtained from all participants.

A questionnaire was completed by all participants that included demographical questions, occupation, duration of the profession, diet (consumption of undercooked meat, standard water, raw milk), daily activity (contact with cat, contact with soil), and questions about previous blood transfusions, previous screening for toxoplasmosis and use of gloves when slicing meat.

2.6. Samples Collection

Human Sample Collection: A sample of up to 3 ml of blood was obtained from each participant using a 5 ml syringe into a plain tube. The serum was separated from the blood by centrifuging and transferred to Eppendorf tubes to measure IgM and IgG. The serum was stored in a freezer at -80°C until used. The serum samples were screened for *T. gondii* using ELISA IgG and IgM.

Animal Sample Collection: Using a plain vacutainer tube, approximately 5 ml of blood was collected from the jugular vein of each animal, including sheep, goats, cattle, and camels after slaughtering. The samples were taken overnight in 4°C and serum separated by centrifugation. The samples were separated in plain tubes and stored at - 80°C until used.

2.7. Laboratory Investigation

IgM and IgG was done by enzyme-linked immunosorbent assay (ELISA) (ARCHITECT plus analyzer, Abbott, Abbott Park, Illinois, U.S.A) according to manufacturing procedures for all samples from humans and animals.

2.8. Data Analysis

Data were analyzed using SPSS software (version 25). The chi-square test was used to compare the serology result frequencies among the different taking a *p*-value of less than 0.05 as the level of significance.

3. Results

3.1. In Human

The present study including a total of 108 workers in the slaughterhouses in the Makkah area. Table (1) shows the total number of slaughterhouses tested for anti-*T. gondii* IgG and IgM by using ELISA. The prevalence of acute toxoplasmosis was 1 (1.5%) in workers and 0 (0.0%) in butchers while the prevalence of chronic

toxoplasmosis was 13 (20%) in workers and 14 (32.6%) in butchers with overall prevalence 27(25.0%).

3.2. In Animals

The current study included 160 animal samples; these samples were collected from the slaughterhouses in the Makkah area in Al-Ka'akya and Al-Muasim. The results obtained confirmed that all the samples from camels, cattle, sheep, and goats had no reaction and showed negative results for IgM and IgG.

3.3. Risk Factors

Socio-demographical Factors: As shown in table (2), the age of participants, nationality, occupation, location and working duration played no role in the risk of acquiring infection (*p*<0.05).

Attitude, Behavior Risk Factors: In table (3), contact with cats has a significant relationship with acquired chronic infection (*p*>0.05). The other risk factors, such as blood transfusion and using gloves do not correlate with getting an infection (*p*<0.05). Some of the expected risk factors, such as eating raw or undercooked meat, drinking water, and drinking unboiled milk showed no differences because all the answers were no.

Table 1: Prevalence of toxoplasmosis in workers & butchers using ELISA IgM & IgG.

Variable	Target Group		Chi ²	P-value
	IgG Positive	IgG Negative		
Occupation				
Butchers	14(32.6%)	29(67.4%)	2.177	0.140
Workers	13 (20%)	52(80%)		
Age group				
20-29	0 (0.0%)	9(100%)	4.792	.0188
30-39	12(32.4%)	25(67.6%)		
40-49	13(26.5%)	36(73.5%)		
50>	2(15.4%)	11(84.6%)		
Location				
Al_Ka'akya	25(25.8%)	72(74.2%)	0.304	0.582
Al_Muasim	2(18.2%)	9(81.8%)		
Duration group				
1-10	4(25%)	12(75%)	5.587	0.232
11-20	19(22.6%)	65(77.4%)		
21-30	3(50%)	3(50%)		
30>	1(100%)	0(0.0%)		
Nationality				
Bangladesh	22(23.9%)	70(76.1%)	6.873	0.076
Egypt	5(55.6%)	4(44.4%)		
Pakistan	0(0.0%)	4(100%)		
African	0(0.0%)	3(100%)		

Table 2: The influence of socio-demographic factors

Target group	ELISA IgM		ELISA IgG	
	Positive	Negative	Positive	Negative
Workers	1(1.5%)	64(98.5%)	13(20%)	52(80%)
Butchers	0(0.0%)	43(100%)	14(32.6%)	29(67.4%)
Total	1(0.93%)	107(99.07%)	27(25%)	81(75%)

Table 3: The influence of Attitude and Behavior risk factors.

Variable	Target Group		Chi ²	p-value
	IgG Positive	IgG Negative		
Contact cat				
Yes	4(66.7%)	2(33.3%)	5.882	0.015
No	23(22.5%)	79(77.5%)		
Blood transfusion				
Yes	1(10%)	9(90%)	1.322	0.250
No	26(26.5%)	72(73.5%)		
Raw meat				
Yes	0(0.0%)	0(0.0%)	-	-
No	27(25%)	81(75%)	-	-
Undercooked meat				
Yes	0(0.0%)	0(0.0%)	-	-
No	27(25%)	81(75%)	-	-
Drinking water				
Yes	0(0.0%)	0(0.0%)	-	-
No	27(25%)	81(75%)	-	-
Unboiled milk				
Yes	0(0.0%)	0(0.0%)	-	-
No	27(25%)	81(75%)	-	-
Use gloves				
Yes	1(100%)	0(0.0%)	3.028	0.082
No	26(24.3%)	81(75.7%)		
Diagnosis				
Yes	0(0.0%)	0(0.0%)	-	-
No	27(25%)	81(75%)	-	-

4. Discussion

The present study was comparable with a study in Mexico done by Alvarado-Esquivel et al., (2011). They were investigating the seropositivity of anti- *T. gondii* IgG and IgM antibodies among workers occupationally exposed to raw meat [18]. One hundred and twenty-four workers occupationally exposed to raw meat and 248 control subjects were examined. Eight (7%) of the butchers and 22 (9%) of the controls were positive for anti-*T. gondii* IgG antibodies. *T. gondii* IgM antibodies were found in five (4%) of the butchers and four (2%) of the controls. This study was also comparable with a study in Iran by Mardani et al., (2015). Their results showed that the seroprevalence of *Toxoplasma* IgG was 41.8% (56 cases) among the group of butchers in Khuzestan and 28.8% in the control group. The seroprevalence of *Toxoplasma* IgM was one case (2.2%) among butchers [19]. The prevalence of human *Toxoplasma* infection varies in different parts of the world, and has been reported with rates up to 75%. The variation of the rate of prevalence may be due to different locations and different weather.

Through the current study and other studies by Alvarado-Esquivel et al., (2011) and Mardani et al., (2015), exposure to raw meat is not related to infection with *T. gondii*. The result of our study suggests the seropositivity with *T. gondii* on workers who had contact with a cat was a statistically significant risk ($p < 0.015$). The results indicated that contact with a cat was the main source of acquiring the infection in workers in the current study and similar to other studies done by Mardani et al., (2015). There was a study in Iraq done by Farah, (2008) that has been compared to the current study. Their study was seroprevalences of *Toxoplasma gondii* antibodies among butchers and slaughterhouse workers in Nenavah Governorate. They determined the specific toxoplasmosis antibodies for 27 serum samples had been collected from butchers and 52 sera from slaughterhouse workers in Nenavah governorate by using the latex agglutination test (LAT) and IgM enzyme immunosorbent assay test (IgM ELISA) [20]. Their result indicated that the rate of *T. gondii* specific antibodies by using latex agglutination test (LAT) in butchers was 59.3% in the slaughterhouses workers. The possible reason for the impact of age on the prevalence of *T. gondii* was that older adults accumulated exposure to different risk factors increased likelihood of the infection with *T. gondii*. In the present study, age showed no correlation with the infection. The results appeared that the main source of infection to the workers in slaughterhouses in Makkah may be in contact with the cat. In fact the actual source of infection may be due to previous handling of meat from infected animals at previous times. Some of the risks, such as blood transfusion, raw meat, drinking water, unboiled milk, working in the garden have shown no significant association with *T. gondii*.

5. Conclusions

The study focussed on *T. gondii* in slaughterhouse workers and reported the presence of chronic toxoplasmosis. The results also showed no infection was registered in different species of animals in both slaughterhouses.

- **No Conflict of Interest**

6. References

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