

## Screening of *Toxoplasma gondii* antibodies and Risk factors among Patients in Asabieh City, Libya

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Accepted 15/06/2019; Received 19/05/2019; Publish Online 25/06/2019

Reviewed By: Dr.  
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### ABSTRACT

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii*. Risk factors include consumption of undercooked meat, raw vegetables, and unfiltered water.

**Aim:** This study aims to screen antibodies titer in toxoplasmosis positive patients in Asabieh city in Libya and to screen risk factors associated with infection in this area.

**Study design:** Data were collected using a cross-sectional design.

**Study Duration and Location:** Data were collected from patients of different ages and genders attending Ali Omar Ascar hospital, Asabieh, during the period of January 2017 to January 2018.

**Methodology:** A single blood sample was collected from 150 patients and spun at 3000 rpm to obtain serum. The serum samples were analysed to detect anti-Toxoplasma IgG and IgM antibodies using enzyme linked immunosorbant assay (ELISA, BioChec) kit according to manufacturer's instructions. A self-structured questionnaire was used to obtain information on farm animal's contact, the process of vegetables washing, meat cooking, Water resource and raw water consumption.

**Results:** The total seroprevalence of toxoplasmosis was 78.6%, out of which IgG and IgM were 68%, and 77.4% respectively. Several risk factors were identified, including daily contact with farm animals (82.6% were +ve,  $P = .0.01$ ), unfiltered water (61.3% were +ve;  $P = 0.003$ ).

**Conclusion:** asabieh area showed a high prevalence of toxoplasmosis, and many environmental risk factors associated with the infection as animal hosts, human lifestyle were also identified, that could help to reduce the risk of spreading and transmission of infection among the populations in the future.

**Key words:** Toxoplasmosis–*Toxoplasma gondii*–Seroprevalence–Risk factors–Asabieh–Libya

### 1 INTRODUCTION

Toxoplasmosis, which is caused by *Toxoplasma gondii*, is one of the most common zoonoses around the world, affecting warm-blooded animals, including humans [1]. It is expected that about one-third of the world's population has been infected with *T. gondii* [2].

Researcher working in North Africa and Brazil around 100 years ago discovered *Toxoplasma gondii*. The parasite has since been found to be capable of infecting all warmblooded animals including humans making it one of the most successful parasitic organisms worldwide. The pathogenic potential of *T. gondii* was recognized in the

1920s and 1930s, in congenitally infected children presenting with the classic triad of symptoms, namely hydrocephalus, retinochoroiditis and encephalitis [3]. *T. gondii* infections involving pregnant women and small ruminants induce abortion or fetal developmental disorders and such infections also pose a high risk in immunocompromised individuals causing severe health problems [4].

People are commonly infected by oral ingestion of water, food, or soil contaminated with oocysts, or by consumption of raw and undercooked meat contaminated with cysts of *T. gondii* [5]. The resist moderate environmental conditions, and contaminate water and soil where they undergo sporu-

lation. The resulting form is responsible for infection via ingestion of either contaminated food, water or dust [6]. Infection of human arise from ingestion of sporulated oocysts, the most important epidemiological route is yet to be determined and the main sources of *T. gondii* infection are diverse among human populations with different cultural and feeding habits [7].

Major routes of infection are: ingestion of oocysts through close contact with infected cat or cat's faeces, ingestion of water or food contaminated with the oocysts, eating raw or under cooked meat from infected animals that contain the tissue cysts, transplantation of infected organs, and congenital infection [8]. Moreover owning cats [9], eating raw or uncooked pork, lamb, mutton, beef, game or mincemeat products [10], eating raw or unwashed vegetables or fruits [11], frequent consumption of raw vegetables outside the home, , having poor hand hygiene , washing kitchen knives infrequently, cleaning the cat litter box, contact with soil , a history of working in soil-related occupations are the common risk factors in toxoplasmosis [11].

*Toxoplasma gondii* has a complex life cycle. It develops an asexual phase in mammals and birds where the parasite cell can adopt an invasive and rapidly dividing form, the tachyzoite, and a latent form which is encysted in the host, the bradyzoite [12]. When members of the felidae family, the definitive host, ingest bradyzoites they differentiate into merozoite, which is an invasive and asexual form that will originate sexual gametocytes, and finally a sporulated form in oocysts, the sporozoite. Consequently, the passage through the different life cycle stages allows the pathogen to adapt to diverse contexts by modulating its virulence and pathogenic potential [13]. Although the stages of the biological cycle of *T. gondii* are categorized, the mechanisms that regulate the transitions between them are not completely understood [14].

Seroprevalence in human populations varies greatly among different countries, different geographical areas within one country, and different ethnic groups living in the same area [15]. In Tripoli, Libya the prevalence of *Toxoplasma* antibodies was found at a titer of 1:16 or above in 51.6% of 2000 adult males, in 43.4% of 300 adult females, and in 43.7% of 1980 schoolchildren [16]. Study on the incidence of *T. gondii* among women from Benjawad region. Blood samples were collected from participants including 280 pregnant and 250 non-pregnant women. Using ELISA Kits tested the samples for anti-*Toxoplasma* IgG and IgM antibodies. Generally incidence rates of *T. gondii* antibodies were 37.17% and 3.58% for IgG and IgM positive, respectively. The study revealed that 37.14% and 3.57% were positive for IgG and IgM anti-*T. gondii* among pregnant women while the incidence rates were 37.20% and 3.60% for anti-*T. gondii* IgG and IgM antibodies among non-pregnant women [17].

Infection of people and animals through contaminated terrestrial and aquatic sources emphasizes the need to jointly examine human, domestic animal, and wildlife populations. The aim of this study is to screen the antibody level in the toxoplasmosis patients in Asabieh Region in Libya and

to investigate the risk factors associated with toxoplasma infected populations.

## 2 MATERIALS AND METHODS

### 2.1 Study Design

The study was a cross-sectional.

### 2.2 Study area

This study is designed to collect data from a total number of 150 patients in Ali Omar Ascar hospital from different age groups, and genders in Asabieh city.

### 2.3 Period of Study

The samples were collected in time period of January 2017 to January 2018.

### 2.4 Questionnaires

A pretested, structured questionnaire was used to assess demographic, socioeconomic, and behavioral variables. Questions focused on possible risk factors for infection, including the presence or ownership of animals, eating habits, and drinking water sources. The questionnaire was adapted from previous studies and WHO recommended sheet. Questionnaire was used was in English language and each patient interviewed personally.

### 2.5 Laboratory Analysis

Blood samples were taken from each patient in this research. Samples were collected aseptically in sterilized 5 ml disposable tubes, then labeled and delivered at 10°C cool containers to the laboratory. The sera were separated by centrifugation for 5 minutes at 3000 rpm and stored at - 20°C until tested.

### 2.6 Enzyme-Linked Immunosorbent Assay (ELISA)

Enzyme linked immunosorbant assay kit was used to detect anti-*Toxoplasma* IgG and IgM antibodies in serum samples. Procedure was performed according to the technique described by BioCheck, Inc. All sera were tested and compared with negative and positive control samples provided with kits by the manufacturer. All specimens during preparation were tested and incubated at 37°C; then measured for absorbance using spectrophotometer ELx800, Bioelisa Reader/Biokit.

### 2.7 Statistical analysis

Data obtained from the serological tests and questionnaire were entered and analyzed using SPSS version 20 statistical package. *P*-values less than 0.05 were considered as statistically significant, using Wilcoxon Signed Ranks Test.

### 2.8 Ethical approval

The study protocol was reviewed and approved by the Ethical Committees of National Authority for Scientific Research (NASR) of Libya in December 2012 by Health Ministry of Libya. All participants endorsed a written informed consent form.

## 3 RESULTS AND DISCUSSION

### 3.1 Seroprevalence of Toxoplasmosis in Asabieh

In this study all admitted patients with toxoplasma from different age group (18-61) were participated and the average age were was 40 years Figure 1. The frequency of toxoplasmosis varies with the age of a subject [18]. In this present study mean ages of participants was 40 years (range=18-61). Our result was different from previous study of toxoplasmosis epidemiological survey in Iran, they found that the level of infection to *Toxoplasma* increasing from childhood, culminating to 30 years of age and gradually decreasing from thereafter. Between the various age groups, the 10-19 years old demonstrated a 50% increase in relative risk to the infection with high antibody titer [19].

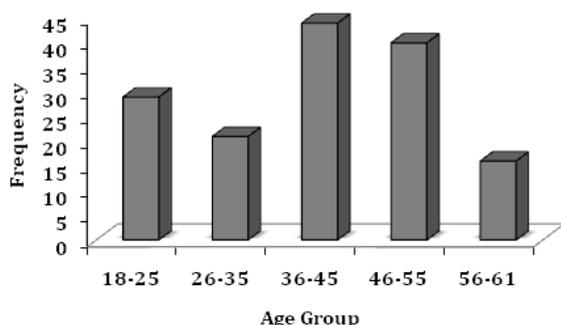


Figure 1. The Frequency of Age Group distribution in the study

In this study the prevalence of toxoplasmosis of 150 blood samples study participant in Asabieh was (78.6%) Table 1. 68% were seropositive for IgG, and 77.4% were IgM positive Table 2. 21.4% of all samples were sero-negative for both IgG and IgM.

Table 1. Sero-prevalence of Toxoplasmosis by either IgG or IgM Anti-toxoplasma Examination by ELISA

Toxoplasmosis	Frequency n	Percentage (%)
Positive	118	78.6
Negative	32	21.4

### 3.2 Risk Factors associated with the infection of Toxoplasmosis in Asabieh

Several risk factors for toxoplasmosis in Asabieh city were identified Table 3. Participants who are daily worker especially who are indirect contact with farm animals were at

Table 2. Results of IgG and IgM Anti-toxoplasma Examination

Category	Frequency(n)	Percentage (%)
IgG	102	68
(+)		
(-)	48	32
IgM	116	77.4
(+)		
(-)	34	22.6

increased risk compared with those who did not have regular contact with them (+ve 82.6%; -Ve 17.33%,  $P < 0.001$ ).

Table 3. Risk factors associated with infection with *Toxoplasma gondii* in 150 patients in Asabieh

Variable	Frequency	Percentage (%)	P value
Contact with farm animals			$<0.01$
Yes	124	82.6	
Never	26	17.33	
Vegetables washing	71	47.3	$<0.001$
Well washing			
Not well washing	79	52.66	
Thoroughly meat cooking	120	80	0.002
Yes			
No	30	20	
Consumption of raw water			0.098
Yes	86	57.4	
No	64	42.6	
Water resource			0.003
Without filtration	92	61.3	
With filtration	58	38.7	

\* Significant with  $P$  value  $<0.05$ .

Consumption of unfiltered water was also identified as a risk factor for toxoplasmosis.

Respondents who did not filter their water were at high risk (61.3%,  $P = 0.003$ ).

Patients with acute toxoplasmosis have a stronger IgG antibody response resulting in high antibody levels soon after infection, and decreasing gradually in approximately 2 yr. IgM antibodies appear during the earliest phases of infection and disappear as soon as in the first or second months in some cases and in most cases by the 6th month after infection [20].

IgM antibodies may appear earlier and decline more rapidly than IgG antibodies and are frequently the first class of antibodies detected after primary infection [21].

Farm animals are thought to play an important role in the prevalence of *Toxoplasma gondii*. Researches have demonstrated that feeding goat's whey to cow and also the presence of a high number of cats were positively linked to *T. gondii* seroprevalence [22]. Several risk factors for toxoplasmosis in Asabieh city were identified, direct contact with farm animals were at increased risk compared with those who did not have regular contact with them.

Humans become infected by ingesting food or water contaminated with sporulated oocysts from infected cat feces

or through ingestion of tissue cysts in undercooked or uncooked meat. Food animals become infected by the same routes, resulting in meat products containing tissue cysts, which can then infect consumers. *Toxoplasma* infection is common in food animals [23]. Toxoplasmosis may occur during the transmission of *T. gondii* via the blood through a broken skin [24]. In this study the way of meat cooking was a significant risk factor as 80% of the population ate well-cooked meat where 20% of them ate undercooked meat ( $p=0.002$ ). As meat from infected animals' increases the possibility of contact with tissue cysts, particularly if no protective equipment, such as gloves, is worn. Additionally, tissue cysts may be ingested during hand-to-mouth contact after handling undercooked meat, or from using knives, utensils, or cutting boards contaminated by raw meat [25]. Therefore the implementation of animal management factors, such as biosecure confinement housing, is important in reducing the levels of infection in animals destined for human consumption [23]. Table 3 shows the Risk Factors associated with the infection of Toxoplasmosis in 150 Patients in Asabieh.

Consumption of unfiltered water was also identified in this Study as a risk factor for toxoplasmosis. Patients who did not filter their water before drinking were at high risk (61.3%,  $P = 0.003$ ). Participants who used non-filtered water were at increased risk of toxoplasmosis compared with those using a filtered water source. Consumption of unfiltered water was from water sources such as rivers, wells, and reservoirs. Oocysts can be transported via rivers and water during periods of heavy rain. Water is not just used for human consumption, but also for washing vegetables and as drinking water for livestock [26]. A previous study in Brazil found that Water was the suspected vehicle of *Toxoplasma gondii* dissemination in a toxoplasmosis outbreak. The Study found also that unfiltered, municipality-treated water was the epidemiologically implicated source of infection for this outbreak [27].

#### 4 CONCLUSION

We have shown that the IgG seroprevalence of toxoplasmosis is high among patients in Asabieh, and have identified some important risk-factors - contact with farm animals, unfiltered water-sources are supposed to be risk-factors of toxoplasmosis in Asabieh. People need to be encouraged to use filtered water and wear protective clothing, such as gloves, when handling animals.

#### 5 COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### 6 CONSENT

All authors declare that verbal informed consent was obtained from the participants for publication of this work.

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