

## The incidence of *Toxoplasma gondii* infection in some patients from Sirte, Libya

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### Abstract

#### Background and objective:

Toxoplasmosis is a widespread infecting disease caused by the coccidian parasite *Toxoplasma Gondii* in humans and other warm-blooded animals. This survey was conducted to show the incidence of infection by the latex agglutination test in some patients of various age groups in Sirt, Libya (LAT). ). We also aimed to find out the possible risk factors of acquiring this infection in Libyan at Sirt region.

### Methods & Materials:

A total of 200 patients were interviewed with questionnaire about known risk factors (age, gender, raw vegetables and fruits intake, source of drinking of water, history of contact with cat and other animals). Blood samples were obtained from some patients (age  $\leq 21$ , 21-25, 26-30 and  $\geq 31$  years) who attended Ibn Sina Teaching Hospital in Sirt City during March, April and May 2013. Blood samples were separated and the serum was stored at  $-20^{\circ}\text{C}$  until analyses. A direct agglutination test was used to detect *T.gondii* antibodies (Kits from human Ref 50023).

### Results:

Out of 200 patients investigated, 91(45.50%) were positive for *T. gondii* infection while 109(54.50%) was negative. There was an increase in infection rate of *T. gondii* with increasing age groups. There was insignificant difference between age groups of patients concerning *toxoplasma gondii* infection rate ( $P=0.05$ ). The toxoplasma infection rate was (35.92 %) in males and (55.67 %) in females, with a significant difference ( $P=0.004$ ). The *Toxoplasma gondii* infection rate was found to have increased due to the use of different drinking water sources in the current study. The highest rate of infection was in patients using untreated water (rainwater) and in patients eating unwashed fresh vegetables or fruit. Cats contact has shown to be an important risk factor for infections with *toxoplasma gondii* ( $P=0.003$ ).

## Conclusion:

The results of this study show that toxoplasmosis is one of most prevalent parasitic diseases in Libya, and it is evident from the results that drinking untreated water and eating vegetables and fruits that have not been washed well before eating are the most common factors increasing prevalence of infection with infection, in addition to living in an environment where cats are present.

## Introduction:

Toxoplasmosis is caused in humans and other vertebrate species by *Toxoplasma gondii*, an apicomplexa protozoan parasite with a heterogeneous life cycle (Petersen *et al.*, 2010 and Tenter *et al.*, 2000). The disease is very prevalent in humans around the world (Lim and Othman, 2014). Both mammal and bird species can be infected by this parasite, members of the felidae (house cat) family are the only known definitive hosts for *T.gondii*, and hot-blooded animals as intermediate hosts (Dubey, 2010). Humans are infected with *T.gondii* by four major pathways: ingesting infected cat oocysts through contaminated food, soil or water, ingesting or handling undercooked or raw meat containing tissue cysts, receiving organ transplants or blood products from donors with acute or latent toxoplasmosis, or, congenitally, transplacental transmission of tachyzoites (Torgerson, 2011).

Most human infections are asymptomatic, but the parasite can often produce devastating diseases (El-Awady *et al.*, 2000). However, severe or even fatal clinical outcomes can occur in infected fetuses and immunodeficient individuals (Ribeiro *et al.*, 2008). *T.gondii* infection in fetal and neonatal may result death or numerous congenital defects during pregnancy, such as hydrocephalus, disorders of the central nervous system, and chorioretinitis and can cause central nervous system manifestations such as Guillain-Barré syndrome (Bossi *et al.*, 1998) or cause brain abscess in immunocompromised patients (Silva *et al.*, 2001). Documented studies of *T. gondii* prevalence rate in Libya was reported by Khader and El Nageh (1987) in Tripoli, who reported 43.4 % in adult males , Setta and Yamani (2008) they reported 43.7 % in school children, 18.14 % in non-pregnant women, 17.6 % in women who reported spontaneous abortion and Mousa, *et al.*, (2011) in Benghazi reported 44.8% infection in patients.

**Aim of study:** Due to the lack of epidemiological toxoplasmosis studies in Sirt City, the objective of this study was to evaluate the incidence of *T. Gondii* infection in some patients of various age groups and studies of the association between infection and certain risk factors for infection.

## Material and Methods:

**Study area:** This study was carried out in Sirt City Libya, located on the Mediterranean Sea on the southern coast of the Gulf of Sidra, at latitudes north 31:12:19 and 16:35:18 latitude west between Tripoli and Benghazi, the city of Sirte, 69 thousand square kilometers.

**Study Samples:** the population of this study was 200 patients (outpatient or inpatient) at Ibn Sina Teaching Hospital in Sirt. Questionnaire forms were required to examine a few of the main risk factors that may affect the incidence of toxoplasma infection among patients, the drinking water, dietary habits and contact with cats. The sample ages were broken down into four ages (<21 years, 21-25 years, 26-30 years, and <31 years, respectively).

**Collection of blood samples:** Blood samples were collected from subjects as 5ml of venous blood was collected under sterile conditions from each individual by medically trained personnel at Ibn Sina Hospital, and then transported to the Microbiology laboratory where the blood samples were centrifuged 3000 r.p.m for 5min to separate the serum, and then store it immediately at-20 C in a single repository until the analysis is completed.

### **Laboratory Assay:**

**Latex Agglutination Test:** the latex agglutination test is relatively rapid and does not require complex laboratory facilities. The latex agglutination test was performed as described in the product information for the Toxo-MT test (Eiken). The agglutination took place in the 96 well microtitre plates bottomed "U" (Tarson). A 0.025 ml (0.2M 2 amino-2 methyl-1 propanol-Hcl buffer solution) buffer volume was applied to each well. And next 0.025 ml of serial serial test diluted from 1:16 to 1:2048 was loaded to each well, followed by 0.025 ml of responsive latex suspension, respectively (Eiken).

The plates were shaken gently and mixed well. Then they were sealed and allowed to remain for 12 h at room temperature. Positive and negative control serums on each plate have also been maintained. A positive reaction was interpreted as an agglutination that spreads uniformly across the well or that which is extreme with irregular edges, whereas a small, distinct circular precipitation in the center suggested a negative reaction (Siamala *et al.*, 2008).

**Statistical analysis:** The Statistical Package for Social Science Version 15 (SPSS), software for biostatic analysis, was used to obtain valid and reliable results from this study. P values (<0.05 were considered to be significant). The data was then presented in tables or figures.

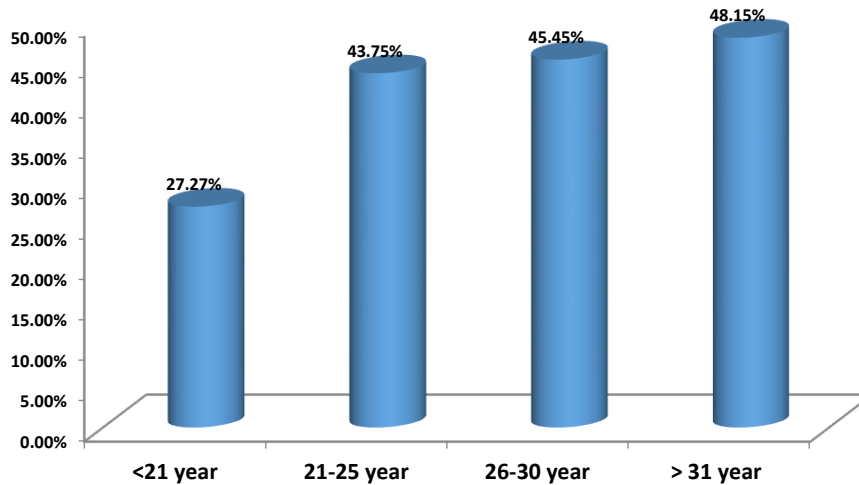
### **Results:**

The current study is one of a series of studies in Libya that assess some behavioral factors that may influence the rate of Toxoplasma gondii infection in Libya.

**The toxoplasma infection rate:** It was found that 91 sera blood samples (45.50 %) out of 200 tested were positive for toxoplasma gondii infection, while 109 sera blood samples (54.50 %) were negative for *T.gondii* infection. (Table1) The results were statistically significant (P=0.000).

**The toxoplasma infection rate according to age groups:** It was clear from the present results shown in Figure (1) that, the sera blood sample showed increase in toxoplasma infection rate

with increase the age groups 3(27.27%), 21(43.75%), 15(45.45%) and 52(48.15%). No important association between toxoplasma infection and patient age groups was observed (P=0.05).



**Figure (1): Toxoplasma infection rate according to age groups of patients**

**Toxoplasma gondii infection rate according to gender (Table 1):** Out of 103 male and 97 female sera blood samples collected from the patients total of 37 (35.92%) and 54 (55.67%) respectively were found to be seropositive for antibodies against *T. gondii* infection. There was a high significant relation between the seropositivity and gender of patients (P=0.004).

**Table (1): Toxoplasma infection rate according to gender of patients:**

		No. of sample examined	No. of positive	No. of negative	Statistical significance
<b>Total</b>		<b>200</b>	<b>91(45.5%)</b>	<b>109(54.5%)</b>	<b>P=0.000</b>
<b>Gender groups</b>	<b>Male</b>	<b>103</b>	<b>37 (35.92%)</b>	<b>66 (64.08%)</b>	<b>P=0.004</b>
	<b>Female</b>	<b>97</b>	<b>54 (55.67%)</b>	<b>43 (44.33%)</b>	

In this study, three variables were identified as possible risk factors associated with toxoplasma infection:

1. ***T. gondii* infection rate according to source of drinking water system:** the present results showed that infection rate of *T. gondii* was typically lower in patients' serum samples, using only treated water (33.33%), compared to patients' sera samples using both treated and rain-water (46.49%), as shown in the (Table:2). No significant correlation existed between the incidence of toxoplasma infection and the source of the drinking water system of the patient. (P=>0.05)

2. ***T. gondii* infection according to eating fresh vegetables or fruits:** Of the (188) patients who eat vegetables or fruits after well-washing and (12) patients who eat vegetables or fruits without well-washing, sera samples obtained from the total of 84 (44.68%) and 7 (58.33%) patients, respectively, were found to be Infected with *T. gondii* (Table 2). There was no significant relation between the toxoplasma infection rate and washed vegetables and fruits before eating by patients (P=0.2).
3. ***T. gondii* infection rate according to contact with cats and domesticated animals:** Among the study population, 77(38.5%) had a history of continuous contact with cats and other animals, 23(11.5%) had a history of continuous contact with cats and 100(50%) had no contact with animals. Toxoplasma infection rate in these groups were 41(53.25%), 14 (60.87%) and 36 (36%) respectively, (Table 2). Showing a statistically significant difference (P=0.003).

**Table (2): *T. gondii* infection rate according to eating fresh vegetables and fruits, contact with domesticated animals:**

Risk factors associated with toxoplasma infection		No. of sample examined	No. of positive	No. of negative	Statistical significance
Total		200	91(45.50%)	109(54.50%)	<i>P=0.000</i>
Source of drinking water	Treated and rain water	185	86(46.49%)	99(53.51%)	<i>P=&gt;0.05</i>
	Treated water	15	5(33.33%)	10(66.66%)	
Eating fresh vegetables or fruits	Well washed	188	84(44.68%)	104(55.32%)	<i>p&gt;0.05</i>
	Un well washed	12	7(58.33%)	5(41.67%)	
Contact with domesticated animals	domesticated cats	23	14 (60.87%)	9 (39.13%)	<i>P=0.003</i>
	domesticated cats and other animals	77	41(53.25%)	36(46.75%)	
	No animals contact	100	36 (36%)	64 (64%)	

## Discussion:

The *Toxoplasma gondii* infection rate in the current study was (45.5%), the incidence of toxoplasmosis was not very different from that observed in other previous studies from different regions in Libya as; (45%), (61.7%) in Tripoli and (47.4%), (50%), (61.7%), (69%) in Benghazi reported by Gashout *et al.*, (2008) ; Elsaid, *et al.*, (2014) and ; Kasse and morsy (1991), Montoya (2004), Elsaid *et al.*, (2014), and Alkateb *et al.*, (2007), respectively. The high incidence of toxoplasmosis may be due to population lifestyle, including: unsanitary feeding habits such as consumption of raw or uncooked meat, consumption of sheep meat primarily as daily lamb in Libya, which has been documented in previous studies with a high prevalence of parasites of toxoplasmosis in sheep from different regions in Libya (EL-Gomati *et al.*, 2008).

frequent consumption of raw vegetables outside the home, having a pet cat at home or living in an area with stray cats living in close proximity, inadequate hand washing after handling contaminated garden soil (Cook *et al.*, 2000; Fayer *et al.*, 2004, Montoya and Liesenfeld, 2004; Nash *et al.*, 2005; Elsheikha, 2008 and Emelia *et al.*, 2010).

On the other hand, these findings were higher than those observed in another studies done in many other regions of Libya (18.14%), (36.84%), (37.75%), (37.16%) stated by (Abu-Setta and Yamani 2008), Nosseur and Saleh (2016), Kassem and Morsy (1991); Swalem and Feturi, (1994); Bader, (2002), AL Ghariane, (2006) and Boshapor and Kassem, (2006) (2015). This large variability can be due to the difference in climatic conditions, where high incidence is correlated with wetlands that are favorable for oocyst sporulation in cat feces compared to less dry hot areas (Afonso *et al.*; 2010). Oocyst survival often improves in moist environments during long periods of heat, staying viable for more than a year in a moist climate (Dubey, 2010).

Regarding the relation between the incidence of toxoplasma infection and certain risk factors, this findings indicates an increase in the rate of toxoplasma infection with increasing age groups, which is consistent with prior studies in Libya, showing analogous results such as Legnain and Prawecka (1983), Kassem and Morsy (1991), Bader (2002), Magrhi *et al.*, (2003), Alkhunfas (2008), Mousa *et al.*, (2011 & 2015). It may be explained by the fact that, due to longer exposure period, older patients are more likely than younger patients to have been exposed to any one of the risk factors. By increasing contact with cats or increasing the likelihood of increased exposure to contaminated vegetable products, these age groups and the consumption of undercooked meat are increasing.

Data from the current study shows that women are at high risk of contracting Toxoplasma infection. In males, the Toxoplasma infection rate (35.92%) was lower than that observed in females (55.67%). This can be explained by the fact that women are much more likely than men to have been exposed to several of the risk factors as a result of a longer exposure period, which is certainly due to many factors: First, women have historically taken more care of pet animals, including cats at home, and second, since they spend more time cooking at home, women handle raw meat more frequently than males. This outcome is consistent with the findings reported by (Yue Xiao *et al.*; 2010 and Pinto *et al.*; 2017). From the other hand, this observation disagrees with the findings of those who have not investigated any difference in the frequency of toxoplasma gondii infection between males and females (Carmen *et al.*; 2006).

Drinking of contaminated water is another source of toxoplasma infection (Bowie *et al.*; 1997), the present results showed high infection rate was observed among patients who drunk untreated water that may have high risk of contamination by oocysts. Corresponding finding was reported by Nosseur and Saleh (2016) and El Said *et al.*; 2014 in Libya, Ishaku *et al.*; (2009) in Niegeria; Krueger *et al.*; (2014) in USA.

Contaminated fresh vegetables or fruit have been reported as a potential cause of Toxoplasma infection. This study found higher rates of infection among patients eating unwashed vegetables or fruit, which may be attributed to contamination by soil and water-borne oocysts.



Coincident studies conducted by (El Said *et al.*; 2014, Da Silva *et al.*; 2014, Cook *et al.*; 2000, Avellino *et al.*; 2004, Nousseur and Saleh 2016). An association has been established between the rate of infection in patients and consuming unwashed fresh vegetables or fruit if proper hygiene and ingestion of oocysts are lacking. Contact with cats and cats excrement have been considered as major risk factors for acquiring infection as oocysts that voided in feces are main source of infection for human, animals and birds. This current result has shown an important association between the rate of toxoplasma infection in patients and cat contact. This result is consistent with many other previous studies done in Libya as; Magrhi *et al.*; (2003), Alkhunfas (2008), Mousa *et al.*; (2011), Malarvizhi *et al.*; (2012), Gamal and Jaroud (2015), Nousseur and Saleh (2016) and Zemene *et al.*; (2012), El Deeb *et al.*; (2012) and Athari *et al.*; (1994). However, the acquisition of cats as pets was not common practice in Libya and most of cats are straying. The soil consider as risk factor for transmitting *T.gondii* due to their contamination by oocysts dispersed in the excrement of stray cats. The later were reported to be more exposed to the parasite than pet ones. Stray cats were found in farms, gardens or may enter houses from time to another, these should increase the chance of infection especially for children living in houses with soil floor or playing in farms during picnics(Lee *et al.*; 2010).*Theoretically, Infected cats play a significant role in contaminating soil; therefore, in order to become infected by this path, people living in a house with soil floors need to have a cat or have contact with it.*

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