



Seroprevalence of *Toxoplasma gondii* infection and associated risk factors among pregnant women in Sebha region, Libya

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ABSTRACT

Toxoplasmosis is one of the most common worldwide parasitic diseases caused by an obligate intracellular protozoan parasite *Toxoplasma gondii*. It is commonly transmitted to humans by accidental ingestion of oocysts voided in cat feces or tissue cyst in meat. Congenital transmission may occur when an uninfected mother acquires primary infection during pregnancy. The acquired infection is usually asymptomatic, while congenital one may lead to abortion, stillbirth or severe abnormalities in the newborn. A total of 190 pregnant women were interviewed with questionnaire about known risk factors (age, residence and occupational status, raw vegetables and fruits intake, source of drinking of water, milk intake, frequency of meat intake, consumption of raw, undercooked meat or processed meat, frequency of eating in restaurant and fast food outlet, contact with cat and soil, history of pregnancy, abortion and blood transfusion). The collected sera were tested for detection of immunoglobulins (IgG&IgM) against *T. gondii* using Enzyme Linked Fluorescent Assay (ELFA) with the VIDAS Toxo-competition kit (BioMérieux, France). A total of 70 (36.84%) out of 190 pregnant women sera were seropositive for *Toxoplasma gondii* specific IgG antibodies, while all examined women were seronegative for IgM. Seroprevalence of *T. gondii* IgG antibodies in respect to previously mentioned risk factors were statistically non-significant ($P>0.05$).

Keywords: *Toxoplasma gondii*, Seroprevalence, Pregnant women, ELFA, Sebha, Libya

INTRODUCTION

Toxoplasma gondii is an obligate intra-cellular protozoan parasite which is widely prevalent in humans, warm-blooded animals and birds throughout the world. It was first described in 1908 by Nicolle and Manceaux in a North African rodent, gundi (*Ctenodactylus gundi*) in Tunisia [1]. It is a tissue cyst forming coccidium functioning in a prey-predator system that alternates between definitive (sexual reproduction) and intermediate (asexual replication) hosts. It is unique among this

group because it can be transmitted not only between intermediate and definitive hosts (sexual cycle) but also between intermediate hosts via carnivorous (asexual cycle) or even between definitive hosts [2]. Sexual reproduction occurs only in felids (domestic and wild cats) after ingestion of cysts present in tissues of an intermediate host resulting in the shedding of oocysts in cat feces that sporulate within few days in the external environment. Asexual development occurs only within intermediate hosts (human, warm-blooded

animals and birds) after ingestion of sporulated oocyst. The sporozoites are liberated and penetrate the intestinal epithelium, where they differentiate into tachyzoites that rapidly replicate by endodyogeny inside any kind of cell and disseminate throughout the body. As a result of the conversion from tachyzoite to bradyzoite, tissue cysts arise as early as 7 to 10 days post-infection and may remain throughout life in most hosts, predominantly in the brain or musculature. Upon ingestion of these cysts by an intermediate host through raw or undercooked meat, cysts are ruptured releasing bradyzoites. The later will infect intestinal epithelium and differentiate back into rapidly dividing tachyzoites for dissemination throughout the body [3].

Toxoplasma gondii can be transmitted via ingestion of raw or undercooked meat, especially pork, lamb, or venison containing *Toxoplasma* cysts [4] or ingestion of unwashed fruits or vegetables that have been in contact with contaminated soil containing infected cat feces [5]. Congenital transmission through placenta (mother to child) may occurs during pregnancy in case of acute phase of infection [6]. Transmission through injection, blood transfusion or organ transplantation is theoretically possible if the donor has recently acquired a *Toxoplasma* infection and is parasitemic at the time of blood sampling [7].

Toxoplasmosis is an important disease and a causative agent that is responsible for abortion in human and farm animals. Primary acquired infection is asymptomatic in more than 80% of immune competent individuals while, the remaining cases may experience fever or cervical lymphadenopathy, sometimes associated with myalgia or other nonspecific clinical signs. Toxoplasmosis is always life threatening in immune compromised patients [4]. Congenital infection results from primary acquired maternal infection during gestation; the severity of fetal infection is inversely correlated, since neonates are asymptomatic in more than 80% of cases when infected during the third trimester of gestation [8]. However, when transplacental transmission occurs during the first trimester, the consequences for fetal development are heavy, often leading to severe abnormalities in the brain and eye tissues or abortion. The abnormalities may include mental retardation, seizures, microcephalus,

hydrocephalus, deafness, and psychomotor deficiency [9].

Several serological procedures are available for the detection of *T. gondii* antibodies in patients, which may aid diagnosis; these include Sabin-Feldman dye test (DT), indirect hemagglutination assay, direct agglutination test, latex agglutination test (LAT), indirect fluorescent antibody assay (IFA), immunosorbent agglutination assay test (IAAT) and enzyme-linked immunosorbent assay (ELISA). The last three tests have been modified to detect immunoglobulin M (IgM) antibodies [10]. In most cases, immunoglobulin M antibodies are detected initially in patients with recently acquired primary infection, but these titers become negative within a few months, while immunoglobulin G antibodies usually appear within 1–2 weeks post-infection and usually persist for life [11]. This study has been designed to determine the prevalence of antibodies to *Toxoplasma gondii* among Libyan pregnant women in Sebha region through detection of immunoglobulins (IgG and IgM). We also aimed to find out the possible risk factors of acquiring this infection.

MATERIALS AND METHODS

The study population included 190 pregnant women during the period from 1st March, 2014 to end of February, 2015. They were interviewed with questionnaire that was designed to assess some of the main risk factors which may influence the prevalence of *Toxoplasma gondii* infection among pregnant women, filled out pertaining each includes socio-demographic items as age, residence and occupational status. Also, it includes dietary and behavioral items as consumption of raw vegetables and fruits, source of drinking water, milk intake, frequency of meat intake, consumption undercooked or processed meat (ham or sausages), eating away from home (in restaurants or fast food outlets), contact with cat and contact with soil (gardening or agriculture). Clinical data as history of pregnancy, abortion and blood transfusion were also included.

About 5ml of venous blood was collected by needle and syringe technique aseptically from each of the pregnant women conducted at Medical Health Centers in Sebha. The blood samples were transported to parasitology department of central medical laboratory where serum was separated

from the whole blood by centrifugation at 3000 rpm for 5 min then labeled and kept at -20°C until use. The collected sera were tested for detection of immunoglobulins against *T. gondii* using Enzyme Linked Fluorescent Assay (ELFA) with the VIDAS Toxo-competition kit ; VIDAS Toxo-IgG-II-ELFA and Toxo-IgM-ELFA for anti-*T.gondii*- specific IgG and IgM antibodies respectively" BioMérieux,France"[12].Statistical analyses were performed using SPSS software. Statistically significant difference was considered if P-value less than 0.05 ($P<0.05$).

RESULTS

A total of 190 pregnant women sera were examined for *Toxoplasma gondii* specific immunoglobulins IgG and IgM antibodies. The obtained results showed that seventy (36.84%) of pregnant women were seropositive for immunoglobulin G (IgG) indicating past-infection .All immunoglobulin G positive cases were seronegative for immunoglobulin M (IgM). Concerning the result of questionnaire; sociodemographic characteristics associated with the risk of *Toxoplasma gondii* infection included age, residence and occupation were assessed. The age distribution of examined and seropositive women showed higher prevalence in the ≥ 30 age group "40%" than in the <30 years age group "30 %". However, women's age showed statically non-significant association ($P=0.211$) with *T. gondii* seopositivity. The sero-prevalence of *T. gondii* was nearly comparable among pregnant women residing in urban"36.77%"and rural"37.14%"areas ($P=0.967$). Concerning seropositivity of *Toxoplasma gondii* among individuals with different occupations; employed pregnant women "37.39%" and non-employed ones "36.00%" had nearly the same prevalence rate ($P=0.846$). (Table 1)

Regarding dietary habits and behavioral characteristics associated with *Toxoplasma gondii* seropositivity; sixteen of the examined women reported to have been a habit of eating less washed raw vegetables and fruits, nine"56.25%"of them were seropositive for *T. gondii*. Most of examined women eat well washed raw vegetables had low prevalence rate "35.06%". However, there was statically insignificant association ($P=0.093$) between habit of eating raw vegetables and *T.*

gondii seropositivity. Concerning source of drinking water ; one hundred and forty three of the examined women drunk untreated water, fifty six"39.16%"of them were seropositive ,while those drunk treated water , fourteen "29.79%" of them were positive for *T.gondii* specific IgG antibodies . Source of drinking water did not demonstrate significant association ($P=0.248$). The seroprevalence of *T.gondii* was higher among women who drunk pasteurized milk "39.51%" than those drinking raw milk"21.43%" , however there was insignificant association($P= 0.067$) between milk intake and prevalence rate. Meat intake may act as potential source of infection for *T.gondii* especially among population having a habit of eating raw or undercooked meat; however this was not the case among people in Libya. On the other hand, one hundred and seventy six women have been frequently consumed meat; sixty five"36.93%" of them were seropositive. Frequency of meat intake was statically insignificant ($P= 0.928$). Intake of processed meat which may be insufficiently cooked increase the rate of seropostivity"47.37%" but it was statically insignificant ($P= 0.133$). Concerning the women eating away from home "in restaurant and fast food outlet", only forty seven of the examined women may frequently eat in restaurant, nineteen "40.43%" of them were seropositive ($P=0.557$) .Contact with domestic cats is often mentioned as a risk factor however, acquisition of cat at homes in Sebha is very rare, only eight of the examined women reported to have cat at homes, of which three"37.5%" were positive for *T. gondii* antibodies. Holding domestic cat at home did not demonstrate significant association ($P=0.969$) with *Toxoplasma* seropositivity. Regarding contact with soil, sixty eight pregnant women have a history of frequent contact with soil especially during picnic in farms or those living in soil floor houses, twenty seven "39.71%" of them were seropositive. History of contact with soil showed insignificant association ($P =0.541$) (Table 2)

Dealing with clinical data and seropostivity, the current study showed an increase of seropositivity among women during second gestation "42.86%". Frequency of pregnancy showed non-significant association ($P= 0.450$) with *Toxoplasma* seropositivity. History of abortion was found to be statically insignificant ($P=0.368$), higher seroprevalence"50%"was observed among pregnant

women having no history abortion compared to those having history of abortion. Also, one hundred and forty five women were found to be aborted during 1st trimester of gestation period, fifty five "37.93%" of them were positive *T.gondii* antibodies. Timing of abortion during gestation was statically non-significant (P= 0.856). The

seroprevalence was high among women having history of blood transfusion "46.67%" in comparison with those having no history of blood transfusion "36.00%". However, there was statically insignificant association (P=0.411) between history of blood transfusion and *T. gondi* seropositivity . (Table 3).

Table (1): Sociodemographic characteristics and seropositivity of *Toxoplasma gondii* among pregnant women

Epidemiological factors		Exam. No	Positive		Negative		P value
			n	%	N	%	
Age	< 30	65	20	30.77	45	69.23	0.211
	≥ 30	125	50	40.00	75	60.00	
	Total	190	70	36.84	120	63.16	
Residence	Urban area	155	57	36.77	98	63.23	0.967
	Rural area	35	13	37.14	22	62.86	
Occupational status	Employed	115	43	37.39	72	62.61	0.846
	Non employed	75	27	36.00	48	64.00	

Table (2) : Dietary habits , behavioral characteristics and seropositivity of *Toxoplasma gondii* among pregnant women

Epidemiological factors		Exam. No	Positive		Negative		P value
			No	%	No	%	
Raw vegetables and fruits intake	Well washed	174	61	35.06	113	64.94	0.093
	Less washed	16	09	56.25	07	43.75	
Source of drinking water	Treated	47	14	29.79	33	70.21	0.248
	Untreated	143	56	39.16	87	60.84	
Milk Intake	Raw	28	6	21.43	22	78.57	0.067
	Pasteurized	162	64	39.51	98	60.49	
Meat intake	Frequently	176	65	36.93	111	63.07	0.928
	Occasionally	14	05	35.71	09	64.29	
Undercooked meat intake	Yes	00	00	00	00	00	-
	No	190	70	36.84	120	63.16	
Processed meat intake (as Burger, etc....)	Yes	38	18	47.37	20	52.63	0.133
	No	152	52	34.21	100	65.79	
Eating in restaurants or fast food outlet	Yes	47	19	40.43	28	59.57	0.557
	No	143	51	35.66	92	64.34	
Contact with domesticated cat	Yes	08	03	37.50	05	62.50	0.969
	No	182	67	36.81	115	63.19	
Contact with soil (Gardening or agriculture)	Yes	68	27	39.71	41	60.29	0.541
	No	122	43	35.25	79	64.75	

Table (3): Analysis of clinical data and seropositivity of *Toxoplasma gondii* among pregnant women

Epidemiological factors	Exam. No	Positive		Negative		P value
		No	%	No	%	

History of pregnancy	First	35	10	28.57	25	71.43	0.450
	Second	35	15	42.86	20	57.14	
	Third	120	45	37.50	75	62.50	
History of abortion	No	10	05	50.00	05	50.00	0.368
	Once	70	30	42.86	40	57.14	
	Twice	60	20	33.33	40	66.67	
Time of abortion	Three times	50	15	30.00	35	70.00	0.856
	1 st trimester	145	55	37.93	90	62.07	
	2 nd trimester	21	07	33.33	14	66.67	
History of blood transfusion	3 rd trimester	24	08	33.33	16	66.67	0.411
	Received	15	07	46.67	08	53.33	
	Don't received	175	63	36.00	112	64.00	

DISCUSSION

The apicomplexan parasite *Toxoplasma gondii* was discovered a little over 100 years ago. Infection with *Toxoplasma gondii* has a worldwide distribution. It is generally assumed that approximately 25 to 30% of the world's human population is infected by *Toxoplasma gondii* [4]. In this study, the overall seroprevalence of *T.gondii* IgG specific antibodies among examined pregnant women was 36.84%. All examined women were seronegative for *T. gondii* IgM specific antibodies. Nearly similar seroprevalence "33%" was reported among normal volunteers women in Tripilo [13]. The seroprevalence of *Toxoplasma gondii* in the current study was lower than that found in other regions of Libya; 63.9%, 48.9-50.1% , 45.8%, 47.4% ,44.8% in Bengazi [14-18] and (69% ,47.4% , 45%,43.4 %)in Tripoli [19-22] .It was higher than that recorded among non-pregnant women "18%" in Tripilo [23].

Concerning the prevalence of *T. gondii* in other countries ,our prevalence was not much different from that given among pregnant women in Iran "38.6 %" [24], Saudia Arabia "35.6%" [25] and Mexico "33.2" % [26]. The sero-prevalence in the present study was low compared to other studies done in Ethiopia "81.1%" [27], Congo "80.3 %" [28], Brazil "68.7%" [29], Egypt "67.5%" [30] ,Sudan "64.1%" [31] , Kuwait "58%" [32], Jordan "54%" [33] , Saudia Arabia of "51.2%" [34] , Morocco "50.6 %" [35] and Iraq "49.2%" [36]. On the other hand, seropositivity of *T.gondii* in this study was higher than studies done in Tanzania "30.9%" [37], Turkey "28.8 %" [38], Philippines "27.1%" [39] and Korea "3.4%" [40]. All examined women were sero-negative for *T. gondii*

IgM specific antibodies; coincident result was obtained by [37] in Tanzania, while variable rates of IgM seopostivity (66.2%, 8.4%, 5.7%, 4.4%, 2.8 %, 2.7%, 2.5 %, 0.45%) were reported by [26, 18, 29, 28, 30, 38, 27], and [24] respectively.

The prevalence vary widely between countries (from 10 to 80%) and often within a given country or between different communities in the same region [41]. This wide variability may be attributed to difference in climatic conditions, where higher sero-prevalence is associated with humid areas which are favourable for sporulation of oocysts that voided in cat feces compared to less dry hot areas [42]. Also, oocyst survival during long periods heat increase in moist conditions, it remains viable in a moist environment for more than a year [43]. The dry hot weather in Sebha may the main reason for low or moderate prevalence of *T. gondii* among pregnant women compared to wet weather in other region and countries. Also, differences in personal hygienic measures and feeding habits may play a role in the great variation of seroprevalence.

Regarding association between IgG seropositivity and studied several risk factors, this study showed an increase in sero-positivity of *T. gondii* antibodies with increasing age, which is consistent with other studies in most countries [13, 18, 29, and 37]. This could be explained by the fact that older women are more likely to have been exposed to any one of the risk factors than younger women as a result of longer exposure time. However a clear declination of seropositivity with age was reported in Saudia Arabia [44] and Tunisia [45]. Nearly comparable seroprevalence among pregnant women resident in rural and urban areas, this might be due to similar style of life in both areas at Sebha region. On contrasting to this

finding, higher prevalence in rural area than urban one was reported by [26] and [37], while residents from urban areas were more infected with *T. gondii* than those from rural areas [46]. There was insignificant difference in seropositivity between employed and non-employed women; this is come in accordance with [27]. However, higher infection rate among employed pregnant women and lower one were recorded by [37] and [26] respectively.

Contaminated raw vegetables and fruits have been reported as a potential source of *T. gondii* infection. This study revealed higher seropositivity among women eating less washed vegetables and fruits, this may attributed to their contamination with oocysts that carried by soil and water. Coincident studies performed by [13, 29, 47, 48] found an association between the seropositivity among pregnant women and intake of raw vegetables if proper hygiene is lacking and ingestion of oocysts occurs. Drinking of contaminated water is another source of *T. gondii* infection [49]. So, high prevalence was observed among pregnant women who drunk untreated water that may have high risk of contamination by oocysts. Corresponding finding was reported by [13] in Libya, [50] in Nigeria; [51] in USA, this was not the case in study done in Tanzania [37] that found high seropositivity in women using pipe water system. Consumption of unpasteurized raw milk does not seem to be a common dietary habit as most of the examined women consumes pasteurized milk, the seroprevalence among those women was high compared to those drunk raw milk. The infection via milk is not an important risk factor as tachyzoites which may pass in milk is very sensitive to environmental conditions and is usually killed rapidly outside the host. Also, tachyzoites were suggested to be rare cause of acquired toxoplasmosis in humans after the consumption of unpasteurized goat's milk [52]. However, a significant association between the consumption of raw milk and infection was recorded [29].

Meat of warm-blooded animals and birds has been considered a major source of *Toxoplasma* infection especially in countries that consumed raw or undercooked meat. Several studies identified an association between eating raw meat and *Toxoplasma gondii* seropositivity [26, 27, 29, 31, 53], although other studies found no association [37, 54]. This was not the case in our study as all examined women consume proper cooked meat and

the habit of eating raw meat among people here is very seldom. This sero-positivity of *Toxoplasma gondii* was high among pregnant women eating roasted mutton or processed meat as hamburger, minced meat and Sharrma which may be insufficiently cooked. Also type of meat consumed should be considered since pork, mutton and farm chickens are commonly infected, while beef are rarely infected [55]. The pregnant women eating in fast food outlet or restaurant showed high seropositivity, this may be explained by poor hygienic measures in these places which give a good chance of infection through less washed vegetables (salad) or inadequately cooked meat.

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. Several studies have concluded that contact with cats increased the risk of *Toxoplasma gondii* seropositivity [27, 30, and 56]; others found no association [17, 57]. In the present study, there is a weak or non-significant relationship between seroprevalence and contact with cats, this finding corroborates with other previous studies done in Libya [13, 18, 20] 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 [58]. 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. The present investigation ensured a positive relationship between contact with soil and the infection, coincident finding obtained by [27] and [31]. Moreover, infected soil may play other role in transmitting infection through contamination of raw vegetables or water during windy weather.

Dealing with clinical data, high seropositivity of *T. gondii* was observed during second time of pregnancy, comparable finding was observed by [26] and [29] among women during third time of gestation and in those having more than six time gestation respectively. Pregnant women having no history of abortion showed high seropositivity, in contrast, [56] reported high seropositivity among women having history of previous abortion.

Transmission of *T.gondii* through blood transfusion is theoretically possible if the donor has recently acquired a *Toxoplasma* infection [7]. High seropositivity was observed among women having

a history of blood transfusion, this come in accordance with some studies [13, 26] and inconsistent with other studies [18].

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