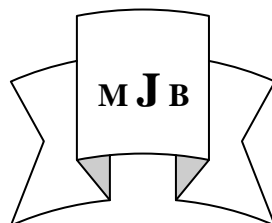


Seroprevalence and Associated Factors of *Toxoplasma* Infection among Sample of Pregnant Women in Wassit Governorate- Iraq

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Abstract

Objectives: Identifying the seroprevalence of acute anti-*Toxoplasma gondii* IgM and chronic IgG antibodies, the environmental and behavioral factors and some socio demographic data of the study sample.

Methods: A descriptive Cross sectional study done on 275 pregnant women (15-45 years old). All the studied women were interviewed and blood samples were taken.

Results: The seroprevalence of anti-*Toxoplasma gondii* IgM antibody (acute infection) was 22.2%, whereas IgG seropositivity (chronic infection) was 32.4%, with significant association with consumption of undercooked meat, undercooked beef consumption, consumption of one meal of meat /day and exposures to soil and /or contact with cats were significant associated factors.

Conclusion: There is a considerable prevalence of *Toxoplasma gondii* infection.

Key words: Toxoplasmosis, seroprevalence, pregnant women

الخلاصة

هدف البحث: تحديد انتشار الإصابة بداء المقوسات القندية والعوامل الاجتماعية، البيئية والسلوكية المرتبطة بها لدى عينة البحث **طريقة العمل:** نوع الدراسة هو وصفية مقطعية لدى عينة من (٢٧٥) امرأة حامل تتراوح أعمارهن (١٥-٤٥ سنة). جمع المعلومات كان بالمقابلة الشخصية واخذ عينة من الدم من عينة البحث.

النتائج: أن النسبة المئوية لوجود الأجسام المضادة من النوع "م"، والتي تدل على نسبة الإصابة الحادة للمرض هي ٢٢,٢% (٦١ سيدة من إجمالي ٢٧٥)، بينما كانت نسبة تواجد الأجسام المضادة "ج"، والتي تعبر عن الحالة المزمنة للمرض ٣٢,٤%

وأن معدل الانتشار المصلي للإصابة الحادة بترابط ارتباطا ايجابيا مع استهلاك اللحوم الغير مطبوخة جيدا، استهلاك لحم البقر الغير مطبوخ بشكل جيد أكثر من لحم الخراف أو الدجاج، استهلاك وجبة لحوم يوميا، التعرض للتربة و / أو التماس مع القطط كانت من عوامل الخطورة التي يعتد بها إحصائيا

الاستنتاجات: هناك نسبة كبيرة من الإصابة الحادة بطفيلي المقوسات القندية.

Introduction

Toxoplasma gondii (*T.gondii*) is an obligatory intracellular protozoan parasite which appears to have broad host specificity. Cats and wild Felines are the only definitive host while all other worm-blooded animals including humans are intermediate hosts. [1]

Up to one third of the worlds human population is estimated to carry a toxoplasmosis infection [2].

The Centers for Disease control and prevention notes that overall seroprevalence in the United States as determined with specimens collected by the National Health and Nutritional Examination Survey (NHANES) between 1999 and 2004 was found to be 10.8% with seroprevalence among

women of childbearing age (15 to 44 years) of 11% [3].

Infection is acquired by ingestion of viable tissue cysts in meat or Oocysts excreted by cats that contaminate food or water. [2] Congenital transmission may occur when an uninfected mother acquires primary infection during pregnancy. [1]

The organism is often transmitted across the placenta to the fetus causing congenital toxoplasmosis. The severity of this infection ranges from spontaneous abortion, prematurity, chorioretinitis and neurological defects to asymptomatic status. [4]

While the prevalence rates of *T. gondii* were up to 50-80% in Central and South American as well as some European populations, primary infection with *T. gondii* in pregnant women occurs all over the world with frequencies between 0.1-1% [5] Serological testing for anti-*Toxoplasma gondii* antibodies is the mainstay for the diagnosis of toxoplasmosis. Diagnosis of acute maternal infection is mainly based on detection of the rise in IgM antibodies level which appear sooner after infection than IgG antibodies and disappear faster than IgG after recovery. [1]

The risk factors that are often associated with acute infection in pregnant women were eating raw or undercooked meat and soil contact. Weaker associations were observed for tasting raw meat during preparation of meals, eating salami, drinking unpasteurized milk and animal contact [1], [2]. Limited studies have been conducted to explore the seroprevalence of *T. gondii* among pregnant Iraqi women (AL-dulemi 2000; Al-jubori 2005, khalil 2007) that reported (20%, 18.3%, and 16% respectively). A prevalence rate of

anti-*Toxoplasma* IgG (25%) and IgM (5%) was reported in the Eastern region. [6]

Material and Methods

A descriptive Cross sectional study was conducted at two major Public Hospitals (Al-Battol for Gynecology and Children and Al-Zahraa Teaching Hospital), in Wassit governorate for the period from October 2009, till the end of February 2010. the target population was pregnant women attending the outpatient clinics of the above mentioned hospitals. The pregnant women were those seeking prenatal care at their first antenatal visit who accepted to participate in the study were included during the study period, 280 pregnant women were attended. Out of the 280 pregnant women, 275 were included in the study, and 5 were excluded because either they did not accept to participate and did not provide blood for analysis or did not submit the questionnaire or blood sample hemolysis after centrifugation and draw. Participants were asked to provide a blood sample and answer a questionnaire by direct interview which contains sociodemographic questions, including age, number of children, occupation as well as questions related to route of exposure to the parasite, and obstetrical history

A non probability (convenient) sample of pregnant women at their first antenatal visit, who accepted to participate in the study.

The sample size was calculated as 240 on a prevalence of 20% (as reported in similar regions), $p=0.05$ at a confidence level of 95%. A total of 20% of the sample population was added to the sample size. So, the final study population size was 275. This study was approved by the Iraqi MOH and the wassit health department the purpose and procedures of the study

were explained to all participants and advice on specific precautions to take to prevent *Toxoplasma* infection, all agreed to participate except five pregnant women did not complete an interview because of contact failure and refusal to participate. The sample selection was a non probability (convenient) sample of pregnant women at their first antenatal visit attending the out patient clinic for antenatal care.

Questionnaire was prepared after review of the available literatures. Face to face, interview instead of self-administrated questionnaire, because some of pregnant women were less than secondary school education and had difficulty in reading.

Five mL of blood sample was obtained from each participant by vein puncture using disposable syringes with needle, transported to non-Hebraized tube, sera were separated by centrifugation at 2000 RPM for 10 minutes, and then stored at -20°C until used, and no preservative was added. The HUMAN TOXO IgG and IgM kit from HUMAN company/ Wiesbaden, Germany were used to analysis by Human Line ELISA system.

Data obtained were entered into a computer database. Statistical package for social science (SPSS version 17) software was used for statistical analysis. Data were recorded as number and percentages. Percentages were compared using the chi-squared test; $P \leq 0.05$ was considered significant. Data were then presented in tables.

Results

The results of the immunoglobulin test for 275 serum samples of pregnant women in Wassit governorate using ELISA test revealed that 148 (53.8%) of them were positive for ELISA screening, 59 (21.5%) for acute Toxoplasmosis (IgM) positive

and 87 (31.6%) for chronic Toxoplasmosis (IgG) positive and 2 (0.7%) for both (IgM & IgG) positive. in comparison with 127 (46.2%) negative for both (IgM & IgG) as show in table (1)

The distribution of anti-Toxoplasma IgM antibody in relation to eating preferences were shown in **Table (2)** the percentage of acute toxoplasmosis among those using rivers as source of drinking water was 4 (36.4%) compared with 57 (21.6%) of 264 using safe water (Treated, from general network such as tap water or bottle water), however this result was statistically not significant ($p = 0.248$).

The highly statistical significant associations demonstrated between the consumption of undercooked meat (not well cooked as Iraqi barbecued kabab and hamburger) 26.4% and those who do not 5.5% ($p = 0.001$).

The statistical analysis results showed that most of pregnant women were asked about the type of undercooked meat consumption the highest percentage (76.0%) were beef consumed out of those (26.3%) were positive for acute toxoplasmosis in comparison with (9.1%) were do not, this finding is statistically significant ($p = 0.003$).

While no statistically significant association was found among those who consumed undercooked lamb meat and positive for anti-toxoplasma gondii IgM antibody (24.2%) compared with (21.1) were do not ($p = 0.556$).

Also, the pregnant women were asked about the consumption of undercooked chicken meat, no statistically significant association was found among those who consumed undercooked chicken meat and positively for anti-toxoplasma gondii IgM antibody (23.4%) and those who do not (20.2%) $p = 0.536$

A significant association was detected for Frequency of undercooked meat consumption ($p = 0.004$) among those consumed one meal /day and positive for anti-toxoplasma gondii IgM antibody (56.3%) and those less than one meal /day (23.8%). Seroprevalence of anti-*Toxoplasma gondii* IgM antibody in relation to soil / cat exposure and traveling abroad were shown in **Table (3)**. The seroprevalence of toxoplasma infection was significantly higher among pregnant women who exposed to soil through gardening or working in agricultural areas (27.5%) of 153

compared with those who did not, 15.6% of 122 ($p = 0.019$).

A highly significant association was found between exposure to cats and seropositivity to IgM ($p = 0.0001$), 39.5% of those who exposed to cats were seropositive to IgM compared to 14.9% of non exposed women.

Pregnant women who had been traveling abroad (outside of Iraq in high seroprevalence area) tended to be at higher chance of getting infected with acute toxoplasmosis (66.7%) of 6 than those who did not (21.2%) of 269 ($p = 0.008$).

Table 1 The seroprevalence rate of anti *Toxoplasma gondii* IgM and IgG antibodies among the studied sample

Anti <i>Toxoplasma gondii</i> Immunoglobulines	ELISA screening (n=275)	
	No	%
Positive for ELISA screening (n= 148)		
• +ve IgM (Acute)	59	21.5%
• +ve IgG (Chronic)	87	31.6%
• +ve IgM+IgG (Acute on chronic)	2	0.7%
Negative for ELISA screening for (IgM & IgG) Seronegative	127	46.2%
Total	275	100%

Table 2 The distribution of anti-*Toxoplasma gondii* IgM antibodies in relation to drinking and eating preferences

Drinking and eating preferences	Total (n=275)		IgM positive ELISA screening (Acute) (n=61)		P value
	No.	%	No.	%	
Source of water					
River	11	4.0	4	36.4	0.248
Safe water	264	96.0	57	21.6	
Undercooked meat consumption					
Yes	220	80.0	58	26.4	0.001*
No	55	20.0	3	5.5	
Eating beef					
Yes	209	76.0	55	26.3	0.003*
No	66	24.0	6	9.1	

Eating lamb					
Yes	95	34.5	23	24.2	0.556
No	180	65.5	38	21.1	
Eating chicken					
Yes	171	62.2	40	23.4	0.536
No	104	37.8	21	20.2	
Frequency of meat consumption(n=220)					
1 meal/d	16	7.2	9	56.3	0.004*
Less than one/d	204	92.8	49	23.8	

*Significant using Chi-squared test at 0.05 level of significance

Table 3 Seroprevalence of anti-*Toxoplasma gondii* IgM antibodies in relation to soil /cat exposure and traveling abroad

soil /cat exposure and traveling abroad	Total (n=275)		IgM positive ELISA screening (Acute) (n=61)		P value
	No.	%	No.	%	
Soil exposure					
Yes	153	55.6	42	27.5	0.019*
No	122	44.4	19	15.6	
Cat exposure					
Yes	81	29.5	32	39.5	0.0001*
No	194	70.5	29	14.9	
Travelling abroad					
Yes	6	2.2	4	66.7	0.008*
No	269	97.8	57	21.2	

*Significant using Chi-squared test at 0.05 level of significance

Discussion

In this study, it was found that among (275) pregnant women selected in two Public Hospitals in Wassit governorate examined by ELISA technique, 148 (53.8%) of them showed **seropositive** result, 59 (21.5%) for acute toxoplasmosis (IgM) Immunoglobulin and 87 (31.6%) for chronic toxoplasmosis (IgG) positive and 2 (0.7%) for both (IgM & IgG) Immunoglobulines. in comparison with 127 (46.2%) negative for both (IgM & IgG) Immunoglobulines (**Table 1**).

Close to be similar results were mentioned by Yacoub et al., (2006) in Basra who recorded (52.1%) [7], Mustafa (2000) found that (52.38%) of 210 pregnant women are infected with *T.gondii* in Al-Tameem governorate. [8]

The seropositive rate in this study was higher than that recorded by Al-Harathi et al., (2006), who recorded a seropositivity rate of (29.4%) for IgG and (5.6%) for IgM among (197) pregnant women in Makkah, Saudi Arabia by using ELISA technique [9], Nijem and Al-Amleh (2009), among pregnant women in Hebron district, Palestine, found (27.9%) for IgG and (17.6%) for IgM seropositivity rate using ELISA technique [10]

While seropositivity rate was lower in the present study than that recorded by Al-Nakib et al. (1983) in Kuwait found the seropositive rate of both antibodies (IgG, IgM) among pregnant women to be (58.2%).[11]

The prevalence of *T. gondii* infection in pregnant women varies substantially among countries and different geographic regions in the same country

could be due to differences in climatic and sociodemographic factors [12]. This may explain the variation in seropositivity

Regarding the drinking and eating preferences of the studied sample a higher seropositive anti-*Toxoplasma gondii* IgM antibodies 4(36.4%) was found among pregnant women using uncontrolled water sources compared with 57(21.6) using safe water [Table 2]. Using rivers as source of drinking water gives more chance of taking water contaminated with the Oocysts of the parasite from infected cat's feces. However this association was statistically not significant.

This result was in agreement with that recorded by Al-Harhi et al., (2006) in Makkah, Saudi Arabia who found no significant association between seropositivity and source of water supply.[9] But in disagreement with that of Ertug et al., (2005) Aydin province, Turkey who recorded higher prevalence among those using general network water, The municipal network water in Aydin is collected to processing pools from open springs next to a few villages, The high seroprevalence in general network water users may be due to the presence of Oocysts in chlorinated network water. [13]

In the current study, there was a highly significant association between IgM seropositivity and undercooked meat consumption (not well cooked as Iraqi barbecued kabab and hamburger) $p=0.001$ (Table 2)

Similar findings have been reported by Fallah et al., (2008) in Hamadan, Islamic Republic of Iran who found consumption of undercooked meat have statistically significant association with higher infection rates [14].The reason of increase seropositivity among pregnant women who consumed undercooked meat may belong to consumption of

barbecued kabab or hamburger which traditionally served as undercooked meat, from an infected animals. But inconsistent with that recorded by Al-Harhi et al., (2006) in Makkah, Saudi Arabia who found no significant associations between seroprevalence of anti-*Toxoplasma* IgM antibodies and consumption of undercooked meat [9]. Cultural differences and the amount and type of meat consumed may explain the variation in seropositivity.

Frequent consumption and type of meat (Pork, beef, and lamb) were identified as the principle risk factor in several recent studies of *T.gondii* infections in humans. In wassit, undercooked beef consumption was greater than that of lamb meat, and Pork is not used by Moslems because of religious ban, and there was a highly significant association between IgM seropositivity and undercooked beef consumption $p = 0.003$ (Table 2).

This result was in agreement with that recorded by Baril et al., (1999) in France who found highly significant association with undercooked beef consumption and seropositivity to *Toxoplasma* IgM antibodies. [15]

The highly significant association of seropositivity to IgM antibody and beef consumption may be explained in that beef consumption is more than lamp meat, and those animals are reared outdoors, which may puts them at greater risk of environmental exposure than animals reared indoors (poultry). In fact, a full answer to this question would require determining the prevalence of *Toxoplasma* cysts in meat before its release to the consumer market.

On the other hand, Morris and Crosson (2004) in Auckland found no relation between seroprevalence and type of meat consumed. [16] This may be due to good farm hygiene and animal not reared out the farm.

While no statistically significant association was found between seropositivity and consumption of undercooked lamb meat.

This result was consistent with that recorded by Ertug et al., (2005) in Aydin province, Turkey. Who found no statistically significant association between seropositivity and undercooked lamb meat consumption [13]. The reason may belong to feeding habits of Aydin pregnant women, most of them (181) prefer eating beef meat while (5) only preferred lamp meat.

But inconsistent with results recorded by Laila et al., (2004) in Jordan who found that infection with *Toxoplasma*, is greater with consumption of lamb than that of beef [17]. Changing in feeding habits among different countries may explain the variation in seropositivity.

No statistically significant association was found between seropositivity and consumption of undercooked chicken meat. Table (2)

This result was in agreement with that recorded by Ertug et al., (2005) in Aydin province, Turkey who found no statistically significant association between seropositivity and consumption of undercooked chicken meat [13]. This may be due to the poultry rear, most of chicken meat consumption gets from poultry farms which could be at lower risk of environmental exposure.

But, disagreement with the results recorded by Kapperud et al., (1996) in Norway who found significant association between seropositivity and consumption of undercooked chicken meat [18]. Difference in sampling method may explain the variation in seropositivity.

Pregnant women were asked about the frequency of undercooked meat consumption, the statistical analysis results showed highly significant association among those who

consumed one meal /day and more than those who consumed less than one meal /day ($p = 0.004$).

This result was consistent with that recorded by Buffolano, Gilbert et al., (1996) in Naples, Italy who found that recent infection was strongly associated with frequency of consumption of cured pork and raw meat (OR: 3.1 95% CI: 1.6-6.0) [19]. The seropositivity was higher among those who consumed one meal /day than those who consumed less than that, since eating contaminated meat is a well known route of *T. gondii* infection, the lower the frequency of meat consumption the lower the risk of infection. [12]

Inconsistent result recorded by Ertug et al., (2005) in Aydin province, Turkey who recorded no statistically significant association between seropositivity and consumption of undercooked meat daily (45.5 %) $p = 0.099$ [13]. This could be due to differences in sample size.

A statistically significant association was found between *T. gondii* seroprevalence and contact with soil through gardening or occupation $p = 0.019$ (Table 3).

These results were in agreement with that recorded by Jumaian, (2005) and Nimri et al., (2004) in Jordan who found a significant association of infection with soil contact. [20]

But in disagreement with that recorded by Kapperud et al., (1996) in Norway who found no significant association of infection with soil contact [18]

The significant association between *Toxoplasma* infection and soil contact in this study may be explained by the fact that most of the studied sample had not used gloves or washed their hands after such exposures.

Other explanation, mothers who have Soil contact through gardening allows contact with infective Oocysts deposited by any recently infected cat.

While Oocysts take one to five days to become infective, they can remain infective in soil for up to one year. [21] Oocysts are often buried in soil along with cat faeces, the buoyancy of the Oocyst allows it to float to the top layer of the soil after rain or by surface water. Any food items or other objects which come into contact with soil may potentially become contaminated with the parasite in its infectious stage. [13] A strong association was found between exposure to cats (out door or indoor) and seropositivity to IgM antibody ($p = 0.0001$).

This finding was in agreement with Esquivel et al., (2007) in Mexico $p = 0.006$. [22]

Disagreement was found with the results recorded by Fallah et al., (2008) in Hamadan, Islamic Republic of Iran ($p = 0.751$) and Ramsewak et al., (2008) in Trinidad and Tobago who found no statistically significant association between seropositivity and contact with cats ($p = 0.163$) [14],[23]. Difference in hygienic habits may explain the variation in seropositivity.

Cats rearing and stray cats are widely spread in wassit governorate, this may increase chance of contact with cats litter or contaminated food or water by parasite Oocysts and this may explain the strong association between seropositivity and exposure to cats.

Statistically significant association was found between seropositivity and traveling abroad (outside of Iraq in high seroprevalence area such as Syria and Iran) $p = 0.008$

These results were in agreement with that of Kapperud et al.,(1996) in Norway who found several patients who acquired *Toxoplasma* infection while traveling abroad in countries with a higher seroprevalence and the association was statistically significant $p = 0.01$. [18]

Conclusions

The seroprevalence rate of *T.gondii* infection is relatively high among pregnant women in wassit, Iraq compared to other countries in the world. A significant association has been found between undercooked meat consumption by pregnant women and acute toxoplasma infection, with higher infection among those taking one meal of meat / daily than those taking less frequently. Undercooked beef consumption associated with higher seropositivity to acute *Toxoplasma* infection than lamb or chicken meat consumption. Soil exposure is significantly associated with acute *Toxoplasma* infection. Acute *Toxoplasma* infection is more among pregnant women with history of contact with cats.

As the rate of seroprevalence is relatively high among pregnant women, it is suggested that ELISA test for toxoplasmosis should be introduced as a routine test for pregnant women in Wassit Hospitals.

Public health prevention campaigns should focus on the appropriate risk factors mainly on eating undercooked meat, soil exposure cat exposure during pregnancy. Further studies to determine the incidence of acute *Toxoplasma* infection and congenital Toxoplasmosis in humans and the prevalence of infection in stray and household cats and intermediate hosts in this region.

Further research is required to determine the cysts viability in meat products and their condition to develop potential primary prevention strategies susceptible to control these risk factors in general population and in pregnant women.

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