

Detection of *Toxoplasma gondii* Antibodies in Persons Referred to Maamon-Dabbagh Health Center for Medical Examination before Marriage, Erbil, North of Iraq

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Abstract

Background and objectives: *Toxoplasma gondii* is a ubiquitous parasite of warm-blooded animals that causes one of the most common parasitic infections in humans. Symptomatic disease is usually uncommon and most of the infections are asymptomatic. The infection may be serious if it's transmitted to the fetus during pregnancy. Prophylactic measures, early detection of the infection and treatment can avoid congenital toxoplasmosis and many long term effects. This study was conducted to evaluate the prevalence of *Toxoplasma gondii* antibodies among healthy persons referred to Maamon-Dabbagh Health Center for premarriage medical examinations.

Methods: A total of 322 serum samples were randomly collected and examined for detection of specific *Toxoplasma gondii* IgM and/or IgG antibodies by Latex agglutination test (LAT). IgM-ELISA and IgG-ELISA (using commercial enzyme-linked immunosorbent assay kit) were also performed to increase the accuracy of the diagnosis. Various information about participants was collected via., questionnaires and data were analyzed using SPSS software11.

Results: The overall prevalence was 29.19% (94/322) using LAT. Seropositive rate of recent (IgM) *T. gondii* infection was 7.76% and latent (IgG) *T. gondii* infection was 19.88%. A significant increase in *T. gondii* prevalence with increasing age was confirmed ($P < 0.001$). No significant relations were observed between anti- *T. gondii* antibodies and gender, occupation, inhabitants of rural and urban areas. Education level and contact with cats.

Conclusions: About 69.57% of women in this study in Erbil City were seronegative. These women are susceptible and are at risk of acquired Toxoplasmosis in their pregnancy period. So health education especially in women who are going to marry and also during pregnancy is necessary to prevent congenital toxoplasmosis.

Key words: *Toxoplasma gondii*, Premarriage, ELISA, Erbil.

Introduction

Toxoplasma gondii (*T. gondii*) is an obligate intracellular protozoan parasite

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with ubiquitously distributed throughout the world¹. It can infect the central nervous system of warm-blooded animals as well as humans².

Usually, *T. gondii* infection does not cause clinical signs in healthy humans³. Serological surveys indicate that about 80% of all primary infections are asymptomatic, due to the effectiveness of the immune system⁴. The tissue parasitism during the proliferative phase may occur without symptoms. It may lead to a transient illness characterized by lymphadenopathy, fever, fatigue, arthralgia, dermatosis, malaise, headache, and myalgia. Latent toxoplasmosis, i.e., the lifelong presence of cysts and anamnestic concentrations of anti-*T. gondii* antibodies in immunocompetent subjects, is considered asymptomatic and harmless⁵.

Most *T. gondii* infections among humans occur in one of following ways: a) by eating raw or undercooked meat containing *T. gondii* tissue cysts b) by ingesting oocysts from soil (for example, through gardening, handling/ eating unwashed vegetables, changing a cat litter box) or from food, water contaminated with oocysts excreted by infected cats. c) by acquiring congenital infection through the placenta. d) by blood transfusion. e) by organ transplantation^{3,6}.

Toxoplasma gondii is an opportunistic parasite that is most dangerous to two populations: immunocompromised patients and fetus whose mothers acquire

acute infection during pregnancy⁷⁻⁸. In immunocompromised individuals, including those with organ transplants, acquired immune deficiency syndrome (AIDS), cancer and in those taking immunosuppressive drugs, primary or reactivated infection can cause life-threatening infections such as encephalitis, pneumonia, chorioretinitis, myocarditis and disseminated infection⁹⁻¹⁰. Acute maternal infection during pregnancy can lead to transplacental transmission causes congenital infection with consequent pathological effects such as mental retardation, blindness and epilepsy¹¹, chorioretinitis, recurrent seizures, hydrocephalus and intracranial calcifications may be present at birth or appear later¹², abortion, microcephaly, hydrocephaly, blindness, calcification of brain cells and even death in uterus^{6,13}.

Seroprevalence data indicate toxoplasmosis is one of the most common human infections throughout the world (Fig 1), however, the prevalence levels reported vary greatly for different countries¹⁴. A high prevalence of infection in France has been related to a preference for eating raw or undercooked meat¹⁵, whereas in tropical areas of Latin America or sub-Saharan Africa where cats are abundant and the climate favors survival of oocysts¹⁶⁻¹⁷. The prevalence of antibodies to this disease shows considerable variation from country to country and between different population groups within a country¹⁸. This variation

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related to various factors such as age, sex, ethnicity, sanitary conditions, geographic climate, nutritional habits, cat or soil contact, patterns of work and behavior,

location and configuration of the community have been main determinant of these dissimilarities¹⁹.

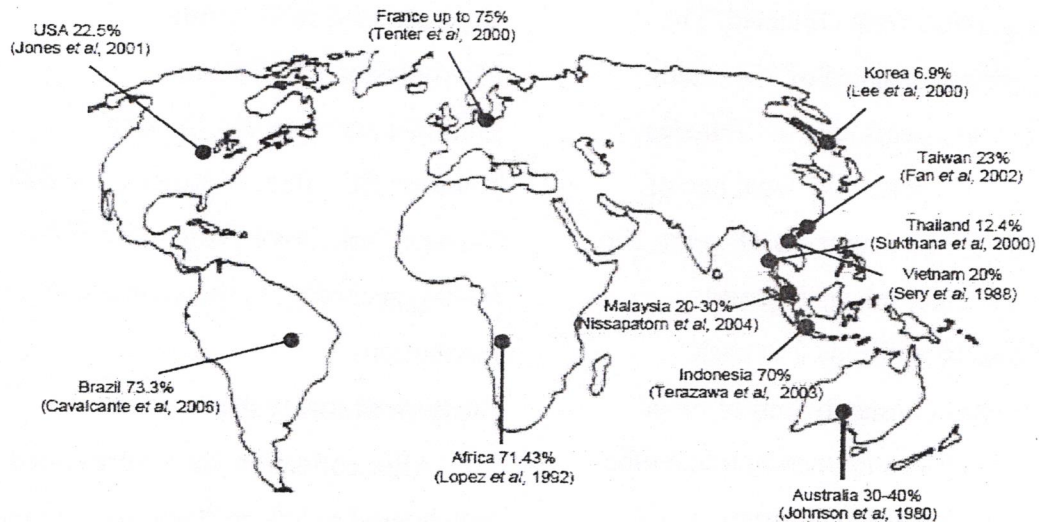


Fig 1—Seroprevalence of *T. gondii* antibodies in healthy persons worldwide²⁰.

Seroepidemiological survey of toxoplasmosis in young girls before marriage and pregnancy will be very useful to prevent congenital toxoplasmosis, because when we know the population of the girls who do not have immunity against toxoplasmosis we can design the preventive measures to prevent congenital toxoplasmosis²¹.

However, no information is known of the seroprevalence *T. gondii* infection in women intending to marry in Erbil City, Northern Iraq. This study was undertaken to determine the frequency of anti-*Toxoplasma gondii* among men and women referred to Maamon-Dabbagh Health Center for medical examinations before marriage, which represent the first trial to explore *T. gondii* in premarriage couples (men and women), as in Iraq

especially and in Kurdistan region there is no information of toxoplasmosis in this group.

Materials and methods

Study population:

The study was conducted on 322 sera collected from males and females referred to Maamon-Dabbagh Health Centre for medical examinations before marriage, in Erbil City, North of Iraq. All of participant appeared healthy, oral consent were obtained from each participants or caretakers after explaining the purpose of the study. Various information about participants such as name, age, gender, type of occupation, place of residence (urban or rural), level of education, then

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they asked about contact with cats or not, were collected through interview.

Serum samples:

From May to August 2011, 322 blood samples were collected. The study group consisted of 161 males aged (18-41) years and 161 females aged (15-37) years (the legal age of marriage in the country is 18 years, for marriage under this age a court certificate is required). For each sample, approximately 5ml of blood was drawn by venipuncture into a tube without anticoagulant. Serum harvested by centrifugation of 3000 rpm of 5 minutes and then sera were aliquoted in several labeled vials, and stored at -20°C until tested²²⁻²³.

Serological tests:

Serum samples were examined to identify the presence of immunoglobulin IgM, IgG. Total IgM and IgG antibodies were examined with latex agglutination test kit (Plasmatec Laboratory Products Unit 29 Dreadnought Trading Estate Bridport Dorset DT6 5BU. U.K.) according to the manufacturer's instructions.

The *Toxoplasma* specific IgM antibodies were detected with commercial kit (*Toxoplasma* IgM Enzyme Immunoassay Test Kit Catalog Number: BC-1087. BioCheck,

Inc 323 Vintage Park Drive Foster City, CA 94404) according to the manufacturer's instructions, while the *Toxoplasma* specific IgG antibodies were detected with commercial kit (*Toxoplasma* IgG Enzyme Immunoassay Test Kit Catalog Number: BC-1085. BioCheck, Inc 323 Vintage Park Drive Foster City, CA 94404) according to the manufacturer's instructions.

Statistical analysis:

After collection, data were coded and entered on the computer using the Statistical Package for Social Sciences (SPSS) version 11 software. the correlation between selected variables and seropositivity was analyzed by Chi-square test. The differences were considered to be statistically significant when the P value obtained was less than 0.05.

Results

To evaluate the seroprevalence of Toxoplasmosis in males and females, who referred to Maamon-Dabbagh Health Centre for premarriage examination, 322 serum samples were examined for *T. gondii* antibodies using LAT. IgM-ELISA and IgG-ELISA were also performed to increase the accuracy of the diagnosis. 94 samples (29.19%) were seropositive and 228 samples (70.81%) were seronegative

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for *T. gondii* antibodies by LAT. 25 samples (7.76%) were seropositive and 297 samples (92.24%) were seronegative for *Toxoplasma* specific IgM antibody. Beside 64 samples (19.88%) were seropositive and 258 samples (80.12%) were seronegative for *Toxoplasma* specific IgG antibody. Moreover, there were no significant differences in seroprevalence of *Toxoplasma* antibody between males and females by LAT and ELISA-IgG. Whereas, we observed a significant difference between males and females by ELISA-IgM ($P < 0.05$) in which seroprevalence in females (11.18%) was high than observed in males (4.35%) (Table 1).

The mean age of the study population was 23.1 years old (range from 15-41 years). The correlation of infection with age showed statistically differences in these settings ($P < 0.001$). there was almost a gradual increases in seropositivity with increasing age, reaching a peak of (44.4%) in the oldest age group (35 years old and over) (Table 2).

Although, there was no significant correlation between seropositivity and the occupation but seropositivity in housewives (33.7%) is considerably higher than individuals who were working outside the home (27.1%) (table 2). 252 (78.3%) individuals of our studies population were resident in urban areas and 70 (21.7%) of these were resident in

rural areas. There was no significant association between the area of residency and seropositivity of *T. gondii*, in spite of, the seropositivity of *T. gondii* antibodies in urban (29.8%) was higher than that rural area (27.1%) (Table 2).

Regarding to educational status, the correlation was not significant between educational status and seroprevalence of *Toxoplasma* antibody ($P > 0.05$). Also we didn't found any correlation between contact with cats and the seroprevalence of *Toxoplasma* antibody, and the correlation statistically was not significant ($P > 0.05$) (Table 2).

Discussion

Toxoplasma gondii is a widely prevalent parasite that is potentially responsible for significant morbidity and mortality in the congenitally infected child and those with immunosuppression and for high morbidity in all persons in the form of ocular disease²⁴. Such women will usually become pregnant early in marriage in our country. Therefore, they are considered a high-risk group for congenital toxoplasmosis, if they seroconvert in the pregnancy period. One of the useful ways to prevent congenital toxoplasmosis is to detect non immune girls before marriage. In this serosurvey (29.19%) was seropositive for *Toxoplasma* IgG and/or IgM antibody by LAT, (27.95%) and (30.43%) were seropositive of males and females respectively (Table

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1). This result was similar to previous results of 25-26, but was higher than 27 and lower than 23, 28-29. No significant difference was observed between males and females, this result is in agreement with results reported in studies done in some other countries^{5, 27, 30}.

The acute phase (IgM) of *T. gondii* antibody was found (7.76%), whereas the latent phase (IgG) was (19.88%). This result is consistent with other previous studies^{5, 22, 31-34}, which they found the percentage of IgM antibodies were less than IgG antibodies. In spite of they differs either higher or lower than our results. Seroprevalence of toxoplasmosis differs throughout the world and between different population groups within a country, these differences might be related to social and cultural habits, geographic factors, climate, nutritional behavior and transmission route⁹, also could be due to use of different serological assays and existence of long years of gap period and different places.

Seroprevalence of toxoplasmosis is known to increase with age^{33, 35-36}. Age effect was observed in the current study and the differences between seropositivity of *Toxoplasma* antibody and age groups statistically was significant, with the high peak level (44.4%) being in 35 years and over age group (Table 2). This result is in accordance with the results of some studies^{5, 26, 32}. On other hand, our result is disagree with other studies^{25, 27, 30} which they observed no relation between

seropositivity of *Toxoplasma* antibody and different age groups. The reason for the rise in seropositivity with age is not clear, although the reason might be the increased possibility of an individual coming into contact with one of the transmission routes as his or her age increases³⁷.

In the current study, no statistical meaningful difference was observed between occupation and *T. gondii* seroprevalence. This result is in agreement with^{28, 32, 38}. Although the high seropositivity rate was observed in housewives (33.7%) (Table 2), and this finding indicated by²⁶ who found highest seropositivity among unemployed women. This result might be explained as a result of more consumption of unwashed or unpeeled vegetables or tasted uncooked meat during cooking or presence of cats in or around the house. Also the high seroprevalence in individuals working out site the home (27.1%), could be explained to exposure to one or more transmission route of *T. gondii* infection, either drinking contaminated water^{32, 39-40} especially *T. gondii* oocysts are highly resistant to environmental influences, including freezing. Oocysts are not killed by chemical and physical treatments currently applied in water treatment plants, including chlorination, ozone treatment and ultraviolet rays³⁹. Or consumption undercooked infected meat (kebabs, hamburger, steak and roast) in sandwich shop and an old restaurant that spread in this city. All mammals and birds that are

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consumed by humans may serve as intermediate hosts for *T. gondii* and, thus, may be a potential source of infection for humans⁴¹. There are several studies established meat samples (beef, buffaloes, sheep, pork, gazelles, oryx, chicken and turkey) from different parts of the world have a higher prevalence for *T. gondii*, thus may act as the single risk factor for human infection⁴²⁻⁴⁷.

The prevalence of toxoplasmosis can vary among different groups, and these differences have also been seen between rural and urban regions. Some studies determined a higher prevalence in rural regions²⁸⁻²⁹, while others did not show any difference between urban and rural inhabitants^{5,32,48}. In our study group, we did not find any significant difference between urban and rural populations, despite the maximum rate of seropositivity was observed in urban regions (29.8%) (Table 2). It might be a result of high presence of stray cats and more consumption of insufficiently cooked meat or raw vegetables in this region.

Like other reports, education has no inverse relation with seropositivity of *T. gondii* infection^{32,49-50}, results of the current study suggested that there was no significant difference between seropositivity of *T. gondii* antibody and level of education. In contrast with other studies which indicated relation between seropositivity of infection and level of education^{26,38,48}.

Although, cats can be an important potential source of transmission of *Toxoplasma* because they are the only hosts that can excrete the resistant *T. gondii* oocysts into the environment⁵¹, and some studies established high prevalence of *T. gondii* infection was found in cats⁵²⁻⁵³. In our study, we did not find any associated between seroprevalence of *T. gondii* antibody and contact with cats, this result is in agreement with results observed by^{22,28}, while other studies observed relation between *T. gondii* seroprevalence and contact with cats^{31,54}.

After a premarriage woman of childbearing age receives a diagnosis of a recently acquired *T. gondii* infection, the question frequently arises as to when they can safely become pregnant, with regard to the risk of congenital transmission of the parasite. It should be understood that there are no definitive data on this subject. Some authors advice has been conservative; others recommend that such women wait 6 months (from the date that the acute infection was diagnosed or documented) before attempting to become pregnant. Each case should be considered separately and preferably, in consultation with an expert⁵⁵.

In the present study, the risk for *T. gondii* infection increased with age and was higher among persons who lived in urban areas. Overall, (69.57%) of premarriage females were seronegative (Table 1), and did not have immunity

against Toxoplasmosis. Therefore this group of population are susceptible and are at risk of acquired toxoplasmosis, and if they acquire the infection during the pregnancy their fetus will be at risk of congenital toxoplasmosis^{29,50,56}.

Knowledge of the prevalence of *T. gondii* antibodies in premarriage women or in a nonpregnant woman of childbearing age group is important because of its relevance to the strategic approach for prevention of congenital toxoplasmosis.

Our recommendation to the health centre institution in Erbil City to design the educational measures for risk of Toxoplasmosis in written form (e.g. books, magazines or simple handbooks), and should be available in different languages. Also including *Toxoplasma* tests in the premarriage examination for identifying the non immune girls before marriage to prevent congenital toxoplasmosis.

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Table 1: Prevalence of *T. gondii* antibodies in individuals referred to Maamon-Dabbagh Health Center for pre-marriage examination by LAT, ELISA-IgM/IgG .

Methods	Seropositive		Seronegative		Total		P value
	No.	%	No.	%	No.	%	
LAT (IgG + IgM)							
Male	45	27.95	116	72.05	161	100	0.24
Female	49	30.43	112	69.57	161	100	NS
Total	94	29.19	228	70.81	322	100	
ELISA-IgM							
Male	7	4.35	154	95.65	161	100	<0.05
Female	18	11.18	143	88.82	161	100	S
Total	25	7.76	297	92.24	322	100	
ELISA-IgG							
Male	35	21.74	126	78.26	161	100	0.70
Female	29	18.01	132	81.99	161	100	NS
Total	64	19.88	258	80.12	322	100	

Detection of *Toxoplasma gondii* Antibodies in Persons Referred to Maamon-Dabbagh Health Center for Medical Examination before Marriage,

Table 2: Prevalence of *T. gondii* antibodies related to risk factors in males and females referred to Maamon-Dabbagh Health Center for premarriage examination by LAT.

Risk factors	No. of seropositive			No. of seronegative			P value
	Male(%)	Female(%)	Total(%)	Male(%)	Female(%)	Total(%)	
Age groups (years)							
15-19 (n= 61)	4(6.6)	14(22.9)	18(29.5)	11(18.0)	32(52.5)	43(70.5)	P<0.0
20-24 (n= 152)	16(10.5)	26(17.1)	42(27.6)	57(37.5)	53(34.9)	110(72.4)	01
25-29 (n= 78)	19(24.4)	6(7.7)	25(32.1)	34(43.6)	19(24.4)	53(67.9)	HS
30-34 (n= 22)	3(13.6)	2(9.1)	5(22.7)	10(45.5)	7(31.8)	17(77.3)	
35&over (n=9)	3(33.3)	1(11.1)	4(44.4)	4(44.4)	1(11.1)	5(55.6)	
Occupation							
Housewife (n=101)	-	34(33.7)	34(33.7)	-	67(66.3)	67(66.3)	1.42
Working outside the home (n= 221)	45(20.4)	15(6.8)	60(27.1)	115(52.0)	46(20.8)	161(72.9)	NS
Residence							
Urban (n=252)	40(15.9)	35(13.9)	75(29.8)	85(33.7)	92(36.5)	177(70.2)	0.182
Rural (n= 70)	5(7.1)	14(20.0)	19(27.1)	31(44.3)	20(28.6)	51(72.9)	NS
Education							
Educated (n=274)	36(13.6)	44(16.1)	80(29.2)	104(37.9)	90(32.8)	194(70.8)	0.00
Non-educated (n= 48)	9(18.8)	5(10.4)	14(29.2)	12(25.0)	22(45.8)	34(70.8)	NS
Contact with cats							
Yes (n=95)	10(10.5)	13(13.7)	23(24.2)	31(32.6)	41(43.2)	72(75.8)	1.61
No (n=227)	35(15.4)	36(15.9)	71(31.3)	85(37.4)	71(31.3)	156(68.7)	NS

الكشف عن الأجسام المضادة للمقوسة الكوندية في الأشخاص الأصحاء المراجعين لمركز مأمون الدباغ للفحص الطبي قبل الزواج، اربيل، شمال العراق

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الملخص:

الخلفية والأهداف: المقوسة الكوندية طفيلي موجود في كل مكان من العالم بوجود الحيوانات ذوات الدم الحار والذي يسبب إحدى أكثر الإصابات الطفيلية شيوعاً في البشر. المرض بوجود الإعراض عادتاً غير شائع وأغلب الإصابات بدون أعراض. ربما يكون المرض خطراً إذا ما انتقل إلى الجنين أثناء الحمل. الإجراءات الوقائية، الكشف المبكر للمرض ومعالجته يمكن أن يجنب الإصابة بداء القطط الخلقي وكذلك يجنب العديد من التأثيرات المرضية على المدى البعيد. أجريت هذه الدراسة لتقييم انتشار الأجسام المضادة لداء القطط بين الأشخاص الأصحاء المراجعين لمركز مأمون الدباغ الصحي للفحوصات الطبية قبل الزواج.

الطرق: جمعت 322 عينة مصل بشكل عشوائي وفحصت للكشف عن الأجسام المضادة للمقوسة الكوندية باستعمال اختبار تلازن اللاتكس. وكذلك اختبار ارتباط الأنزيم للادمصاص المناعي لزيادة دقة التشخيص. جمعت بيانات مختلفة عن المشاركين عن طريق ورقة استبيان. ثم حلت النتائج باستعمال برنامج التحليل الإحصائي.

النتائج: كانت الإصابة الكلية 29.19% (322/94) باستعمال اختبار تلازن اللاتكس ومعدل الإصابة المصلية الحديثة كانت 7.76% والإصابة الكامنة بالمقوسة الكوندية كانت 19.88%. أكد زيادة معنوية لانتشار المقوسة الكوندية بزيادة العمر ($P > 0.001$). ولم تلاحظ أي علاقة معنوية بين الأجسام المضادة للمقوسة الكوندية والجنس- المهنة- السكن في الريف أم المدينة- مستوى التعليم والاتصال بالقطط.

الاستنتاجات: حوالي 69.57% من النساء في هذه الدراسة في اربيل كن ذوات مصول سالبة (غير مصابات). وهؤلاء النساء يكن حساسات وتحت خطر الإصابة بداء القطط المكتسب في فترة الحمل. لذا التعليم الصحي وخصوصاً للنساء اللواتي سيتزوجن وأيضاً أثناء الحمل ضروري لمنع الإصابة بداء القطط الخلقي.
الكلمات الدالة: المقوسة الكوندية - قبل الزواج - اختبار ارتباط الأنزيم للادمصاص المناعي- اربيل.