Serological and microscopical detection of *Toxoplasma gondii* in Kirkuk city- Iraq.

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Abstract

Toxoplasma gondii is an obligate, intracellular, parasitic protozoa that causes toxoplasmosis. Found worldwide, T. gondii is capable of infecting virtually all warm-blooded animals [1]. In this study, the incidence of Toxoplsma in human and stray cats were studied. One hundred and two serum samples from married and non married women were examined by ELISA and LATEX tests in Kirkuk city from July to December 2012. The results showed that the ELISA test is significantly ($P > 0.05$) more sensitive than LATEX, with the rate of 36.53 % positive samples for ELISA compared with 21.15% by LATEX test. The most age group which was infected by Toxoplasma in married women was 34-37 years followed by 30-33years, while the most age group which was infected with the parasite in none married women was 13-16 years. The positive blood groups women appears to be significantly ($P > 0.05$) more at risk for getting toxoplasmosis especially A+(66.6%) and AB+(60%) blood groups. Toxoplasmosis was significantly ($P > 0.05$) more prevalent among married pregnant women (34.2) comparing with married non pregnant women, and the rate of aborted women among them were 25%. High rate of them had two (57.1%) to three (40%) times abortion. Among 30 Toxoplasma positive samples, 10 (33.3%), 8 (42.1%) of them had cats at residence when examined by ELISA and LATEX respectively. The significantly ($P > 0.05$) antibody positive samples were for IgG with rate of 85.5 and 81.8 % in ELISA test for married and none married women respectively, comparing with those samples having IgM

and those having both IgG and IgM. Most of the sample examined by LATEX had low titration (1/10, 1/40) in both married and non married women. Fifty stray cat fecal samples were examined microscopically by compound light and fluorescent microscope to detect the oocyst stage, 6 samples were positive with rate of 12%.

Keywords: Toxiplasma gondii, Serology, Microscopy, Kirkuk, Iraq.

الكشف المصلي والمجهري لطفيلي المقوسات الكوندية في مدينة كركوك-العراق

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الخلاصة

المقوسات الكوندية هي طفيلي داخل خلوي اجباري وحيد الخلية تسبب مرض داء القطط . يكون ذات انتشار عالمي و قادر على اصابة كل الحيوانات ذوات الدم الحار. الدراسة الحالية تناول نسبة انتشار داء المقوسات في الانسان و القطط الشاردة. اختبر مئة و اثنان عينة مصلية من نساء متزوجات و غير متزوجات بطريقة الاليزا واللاتكس في مدينة كركوك من شهر حزيران الى كانون الاول لسنة 2012 . اظهرت النتائج بان طريقة الاليزا حساسة وبصورة معنوية ($P > 0.05$) اكثر من اللاتكس, و بنسبة 36,53% عينة موجبة لاختبار الاليزا مقارنة مع 21,15% باختبار اللاتكس. المرحلة العمرية الاكثر اصابة في النساء المتزوجات كانت 34-37 و 30-33 بينما في النساء غير المتزوجات كانت 13-16 سنة. النساء ذوات فصائل الدم الموجبة اظهرن معنويا ($P > 0.05$) نسبة اصابة اعلى و خاصة فصيلتي A+ (66,6%) و AB+ (60%) .كان داء المقوسات معنويا ($P > 0.05$) اكثر انتشارا (34,2%) بين النساء المتزوجات الحوامل منه في النساء غير الحوامل (25%). نسبة النساء المجهضات كانت (25%) و نسبة عالية (57,1%) منهن كانت لديهن حالتي اجهاض و (40%) منهن عانين من ثلاث حالات اجهاض. من مجموع ثلاثين عينة موجبة للمقوسة الكوندية عشرة نساء (33,3%) , 8 نساء (42,1%) كانت لديهن قطط في المنزل لكل من فحص الاليزا و اللاتكس على التوالي. الاجسام المضادة نوع IgG اظهر زيادة معنوية ($P > 0.05$) بنسبة 89,5 و 81,8% بفحص الاليزا في النساء المتزوجات وغير المتزوجات على التوالي مقارنة بالعينات الموجبة لاجسام IgM و IgG مع IgM . معظم العينات المفحوصة بطريقة اللاتكس كان له معيار واطيء (1/10, 1/40) لكل من النساء المتزوجات و غير المتزوجات . تم فحص خمسون عينة براز للقطط بالمجهر الضوئي المركب و مجهر التالق الضوئي للبحث عن الاكياس البيضية , ست عينات كانت موجبة و بنسبة 12%.

الكلمات المفتاحية: المقوسات الكوندية, الكشف المصلي, المجهري, كركوك, العراق.

Serological and microscopical detection of Toxoplasma gondii in Kirkuk city- Iraq.

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Introduction

Toxoplasmosis is a zoonotic protozoal disease caused by tissue parasite, Toxoplasma gondii. Toxoplasma is a parasite of cosmopolitan distribution present in hot, humid countries able to develop in a wide variety of vertebrate hosts. Cats and other members of Felidae are the definitive hosts, while human and wide range of animals, birds and rodents act as intermediate hosts [1]. Parental infections account for 2% to 3% of all congenital Toxoplasma abnormalities [2]. The transmission of the disease to human is either by eating raw or uncooked meat, blood transfusion and organ transplantation or through ingestion of oocysts introduced into the environment by cats or congenitally during pregnency [3]. Primary maternal T. gondii infection during pregnancy is frequently associated with its transmission to the fetus [4]. The transmission rate of maternal infection to the fetus is estimated to be about 45%; of these, 60% are sub-clinical infections, 9% resulting in death of the fetus and 30% have severe damages such as hydrocephalus, intracerebral calcification, chorioretinitis and mental retardation[4,5]. In most cases, the laboratory diagnosis of acute and latent toxoplasmosis relies on the detection of T. gondii specific IgG and IgM antibodies. And the avidity test of T. gondii specific IgG antibodies has also been very helpful in the diagnosis. Many serological tests such as the LATEX agglutination test, ELISA, indirect fluorescence antibody test (IFA) and hemagglutination test have been used for the detection of antibodies against T. gondii in pregnant women [6,7].

Recent epidemiological studies have identified risk factors for *T. gondii* infection: owning cats being in proximity to seropositive, cats in farming areas, cleaning the cat litter box [8, 9]. In humans, it is one of the most common parasites, serological studies estimate that up to a third of the global population has been exposed to and may be chronically infected with *T. gondii*, although infection rates differ significantly from country to country [3]. The parasite prevalence in Iraq was 36.6% in Kirkuk, 29.2% Tikrit, 58% in Basrsh in 2011 [7,10,11]. Infection in humans and animals is necessary to be evaluated, especially in married pregnant women and women about to marry to prevent the future complications that may occur, therefore the aim of this study was to detect *Toxoplasma* prevalence among human population (married and non married women) and cats by using serologic and microscopic methods.

Materials and methods

Population study

From the 1st of July to the end of December 2012, a cross-sectional study was done for detection *Toxoplasma* antibody, in Kirkuk province in Kirkuk and Azadi Teaching Hospitals. A total of 102 females were chosen whom they were attended to the two hospitals. A questionnaire form was given to each female which include: age, address, blood group, Rh, number of abortion, and cats at residence.

Samples collection

Venous blood (5ml) was drawn carefully and transferred into disposable plan tube, the specimen was left for 15-30 min. then centrifuged at 300 rpm for 5min. to separate clear serum, the sera were tested, if they delayed they were kept at (-20˚C) till use.

Toxocell Latex (bio kit company Linear Chemicals Barcelona, Spain):

Toxocell LATEX is a one step rapid LATEX particle agglutination test or slide for qualitative and semi quantitative determination of *Toxoplasma* antibodies in serum.

Qualitative Test and Semi-quantitative Test: the serum samples is either reacted directly with *Toxoplasma* antigens for qualitative test or with diluted serum for semi-quantitative test. By using Toxocell LATEX (bio kit company Linear Chemicals Barcelona, Spain).

Detection of anti – *Toxoplasma* IgG and IgM by ELISA:

Sera tested by Enzyme Linked Immunosorbent Assay for detection of *T. gondii* infection by evaluating anti –Toxoplasma IgG and IgM . Anti – Toxoplasma IgG and IgM detected by using ELISA kit of Euroimmune company from Germany

Cat fecal examination:

Samples collection: Stray cat fecal samples were collected from general gardens and moist soil from different regions in Kirkuk city. The samples were brought to the laboratory in a clean caps with lids.

Wet mount technique: The fecal sample was examined in its wet state by simply placing a cover slip over the drop of wet fecal material which was made by saline. The slide was examined by the 40X objective using compound light microscope. The wet mount

examination was confirmed by using fluorescent microscope instead of compound microscope.

Formalin- ether concentration technique was performed for some samples.

Statistical analysis: For finding the differences according to different parameter, chi-square (χ^2) test was used for statistical analysis of these samples by statigraphic program. P-values of 0.05 or less were considered statistically significant.

Results

This study was conducted to evaluate *Toxoplasma* prevalence among married and non married women in Kirkuk city, the result of the study (table1) showed that the most age group which was infected with the parasite was 34-37years, 30-33years and 18-20 years, with rate 50, 42.85% and 40% respectively, while the lowest age group was 26-29 years with rate of 28.57% by ELISA kit, fig. 1 shows the positive results.

Fig. 1 *Toxoplasma* ELISA positive wells and *Toxoplasma* LATEX positive and negative results

Table1: Distribution of *Toxoplasma* by ELISA according to age group in married women.

Age group	No. of samples examined	+ve samples	%	-ve samples	%
18-21	5	2	40	3	60
22-25	17	6	35.29	11	64.7
26-29	14	4	28.57	10	71.42
30-33	7	3	42.85	4	57.14
34-37	6	3	50	3	50
38-41	3	1	33.33	2	66.6
total	52	19	36.53	33	63.46

Table 2 indicates that the most age group which was infected with the parasite by LATEX test (fig 1) was 34-37, 38-41 years and 30-33 years, with rate of 50, 33.3% and 28.57% respectively. The lowest rate7.14% was for 26-29years.

Table 2: Distribution of *Toxoplasma* by LATEX according to age group in married women.

Age group	No. of samples examined	+ve samples	%	-ve samples	%
18-21	5	1	20	4	80
22-25	17	3	17.64	14	82.35
26-29	14	1	7.14	13	92.85
30-33	7	2	28.57	5	71.42
34-37	6	3	50	3	50
38-41	3	1	33.3	2	66.6
total	52	11	21.15	41	78.84

The prevalence of infection in non married women which was detected by ELISA showed in table 3, the high rate 30.76% was in 13-16 age group. followed by 26.66% rate in 22-25 years . ELISA test were significantly ($P > 0.05$) more sensitive than LATEX test the rate of positive sample by ELISA test were 36.53, 22% for married and non- married women respectively, comparing with that for LATEX 21.15, 16% for the same groups respectively.

Table3: Distribution of *Toxoplasma* by ELISA according to age group in non- married women.

Age group	No. of samples examined	+ve samples	%	-ve samples	%
13-16	13	4	30.76	9	69.23
17-21	19	3	15.78	16	84.21
22-25	15	4	26.66	11	73.33
26-29	3	0	0	3	100
Total	50	11	22	39	78

The results detected by LATEX test in non married women shown in table 4, high rates 23.07, 15.78, 13.33% in 13-16, 17-21, 22-25 age groups were detected respectively for each group.

Table 4: *Toxoplasma* prevalence by LATEX according to age group in non-married women.

Age group	No. of samples examined	+ve samples	%	-ve samples	%
13-16	13	3	23.07	10	79.92
17-21	19	3	15.78	16	84.21
22-25	15	2	13.33	13	86.66
26-29	3	0	0	3	100
Total	50	8	16	42	84

Regarding the blood group antigens, table 5 shows that the positive blood group antigens is more reliable to infect with *Toxoplasm*a than negative one, especially A, AB, B- blood groups with rate of 66.6, 60, 33.3% respectively for each blood group.

Table5:Relationship between the blood group antigens and *Toxoplasma* prevalence by ELISA test.

Blood group	No. of samples examined	No.of positive samples	%	No.of negative samples	%
A	9	6	66.6	3	33.3
A-	3	0	0	3	100
B	20	3	15	17	65
B-	6	2	33.3	4	66.6
AB	15	9	60	6	40
AB-	12	1	8.33	11	75
O	35	9	25.71	26	74.28
O-	2	0	0	2	100
Total No.	102	30		72	

Most of the positive samples among married women were those samples which belong to pregnant women table 6. The frequency rate was 34.2% of 36.53% for total married women by ELISA. Among pregnant women high frequency rate of *Toxoplasma* was for two and three times aborted women, with rate of 57.1, 40% for case resoectively by ELISA. The same results were noted by LATEX test table 6.

Table 6: Distribution of Toxoplasma by ELISA and Latex in married women in association with pregnancy and number of abortions.

		ELISA				LATEX			
		+ve sample	-ve sample	% of +ve samples	total	+ve sample	-ve sample	%of +ev samples	Total
Pregnant		12	23	34.2	35	7	28	20	35
Aborted women		7	21	25	28	4	24	14.3	28
Number of abortion	One time	1	15	6.2	16	1	15	6.3	16
	Two time	4	3	57.1	7	2	5	28.5	7
	Three time	2	3	40	5	1	4	20	5

Among 30 positive samples belonged to married and non married women examined by ELISA 10 of them had cats at residence (33.3%), while 8 samples (42.1%) were recorded by LATEX test, table7.

Table 7: *Toxoplasma* prevalence by ELISA and LATEX method in married and non-married women in association with cats at residence.

	ELISA			LATEX		
	Positive sample	Cats at residence	%	Positive sample	Cats at residence	%
Married	19	6	31.6	11	5	45.4
Non married	11	4	36.3	8	3	37.5
Total	30	10	33.3	19	8	42.1

IgG and IgM rate detected by ELISA kit in married and non-married women could be noticed in table 8, which shows17 (89.5%) positive married and 11(81.8%) positive non-married women have IgG antibody, while IgM was present in one married and one non married woman only.

Table8:IgG and IgM rate detected by ELISA kit in married and non-married women

	Total no. examined	+ve no.	Type of antibody					
			IgG	%	IgM	%	IgG&IgM	%
Married	52	19	17	89.5	1	5.3	1	5.3
Non-married	50	11	9	81.8	1	9.09	1	9.09

The titration rate detected by LATEX test in married and non-married women table 9, showed that the highest rate were for 1/40, 1/10 titers, in both married (45.4,18.1%) and non-married (37.5, 50%) women.

Table 9 Titration rate detected by latex test in married and non-married women

	Total No. examined	+ve No.	Titrations							
			1/10	%	1/40	%	1/80	%	1/160	%
Married	52	11	2	18.1	5	45.4	2	18.1	2	18.1
Non married	50	8	4	50	3	37.5	1	12.5	0	0

Among 50 stray cat fecal samples examined microscopically by compound light fig.2 and fluorescent microscope fig.3, 6 samples were positive with rate of 12% .

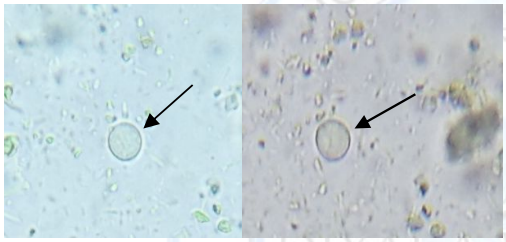

Fig.2 *Toxoplasm* anstained unsporlated oocysts in cat feces (wet mount 1000x) compound light microscope. Arrows ahead.

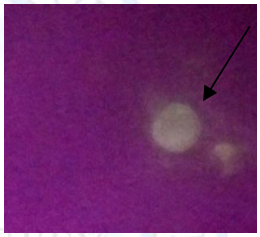

Fig. 3 *Toxoplasm* un stained un sporolated oocysts in cat feces (wet mount 1000x) fluorescent microscope. Arrows ahead.

Discussion

This study was designed to evaluate the prevalence of toxoplasmosis in married and non-married women in Kirkuk city which is detected by ELISA and LATEX test. The prevalence of *Toxoplasma gondii* in married women was 36.53%, this result is identical with [12] which he detect a rate of 38.9%, and with [7] who record a rate of 36.6% but [10,11] recorded rates of 29.2, 58% respectively. Regional variations in the incidence of *Toxoplasma* infection rates from one country to another or even within the same country, has been well documented [3]. This variation has been attributed to climate, cultural differences regarding hygienic and feeding habits [13,14] The prevalence of the parasite among non- married women was

21.15%. Higher rates 58, 34% were reported by others [11,15]. These results espouse the importance of *Toxoplasma* test prior to marriage, in addition to perform other tests. Traditionally, screening for toxoplasmosis has been carried out in France [16] and Brazil [17].

This study reveals that the most age group infected in married women was 34-37 years old. Highest rate of *Toxoplasma* seropositivity was found among the age group 19-35 years (38.3%) [7]. Comparable to our result is that stated by Rabiee [12]. A significant ($P > 0.05$) relation showed in a current study between *Toxoplasma* prevalence rate and the mother's age [18], confirms the fact that seroprevalence of *Toxoplasma* is well known to increase with age; the greater the prevalence, the earlier the rise [16,17].This might be explained by the older the person the longer time being exposed to the causing agent and may retain a steady level of anti-*Toxoplasma* IgG in serum for years.

The effect of ABO blood group antigens on the distribution of *Toxoplasma* in the present study is reveal that the A, AB blood groups were the most blood group infected with *Toxoplasma*. Previous studies investigated possible relationships between the ABO blood group system and the presence of anti-*T. gondii* antibodies, but their conclusions are conflicting. Four studies reported an association between infection by this parasite and B and AB blood groups [19 - 22]. These studies proposed that the B antigen could act as potential receptor for *T. gondii*. However, two other similar investigations did not find any evidence of this association [23,24]. The IgG antibody rate which is detected in this study by ELISA in infected married women was 89.5%. Lower rate was recorded by youssefi they stated a rate of 63.9%.[25] but the rate found by Studeničová [26] was 24.4%. The IgG antibody present in non-married women was 81.8%.

In the present study the rate of IgM was 5.3 % in married and 9.09 % in non- married women. This was comparable with that studied by Al-Hindi and Lubbad [27]. In study of Hajsoleimani [6] the prevalence of recently acquired infections (IgM positive) was relatively low (1.4%).The IgG seropositive rate in his study increased with age. Women older than 30 years had a significantly ($P > 0.05$) higher seroprevalence (48%) compared to those who were 20 or less (28.7%) [6]. IgM antibodies are detected early in the acute infection. Because they may persist for prolonged periods, IgM antibodies may be detected in pregnant women

who were infected in the distant past and before gestation. Therefore, a positive (or equivocal) IgM test result should be followed by confirmatory testing at a *Toxoplasma* reference laboratory [28].The most titration rate which was obtained by LATEX test in both married and non-married women in this study was low (1⁄40) titer. Similar low titers 1/16 were recorded by others [7, 29].

The ELISA seroprevalence of toxoplasmosis in pregnant women in the present study was 34.2%. Hajsoleimani [6] recorded about 37.8% and Al-Harthi [18] recorded about 29.4%. Accordingly this study also directed to aborted women and the result was 25% of cases had positive result and about 57.1% of aborted women had two time abortion. This finding was in agreement with Al-Maqdisy [30] who found that women with two abortions have higher percent of seropositivity by using LATX (34.14%) and also in agreement with Al-Doski [31].

Distribution of *Toxoplasma* seropositivity among patients according to cats contact was studied too, the seropositivity among those having cats at residence was higher than those not having, by both ELISA and latex in both married (31.6, 45.4%) and non married (36.3, 37.5%) women. Somehow identical result was reported by [7], which he recorded about 22.85%. The distribution of disease might be due to cats which get infection from eating birds, rodents, and aborted fetus in slaughter house and shedding their oocysts which lead to contamination of gardens, animal food stuff, granaries and thus lead to infection.

Among fifty stray cat fecal samples examined in this study, the presence of the oocyts were detected in 6 samples only with rate of 12%. Other studies [32,33] have detected oocyst shedding prevalence of much greater magnitude. Jokelainen [32] found that 23% of cats were shedding oocysts in a study of Costa Rican cats, and 17% of fecal kittens in southern Germany contained *T gondii*–like oocysts [8]. 22.6% of Arminian cats were seropositive for the disease [9]. *T. gondii* infection rates in domestic cats vary widely depending on the cats' diet and lifestyle. Although light microscopy following fecal flotation is commonly used to detect *T gondii* oocysts in feces, it is less sensitive than mouse bioassay. The results of our experiment indicated that microscopy is likely to detect moderate to heavy oocyst shedding by cats, but could miss low quantities of oocysts shed towards the end of the patent period.

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