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Seroprevalence and some Demographic Factors Associated with *Toxoplasma gondii* Infection among Male Population in Duhok Province/Iraq

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Abstract:

The present study aims to investigate the seroprevalence rate of *Toxoplasma gondii* infection and its relation to some demographic factors among males in Duhok province/Iraq. A total of 424 random blood samples were collected from the male population of different ages (18-60) years and different social-economic classes. Out of 424 samples examined, 108 (25.47%) were seropositive to the anti- *T. gondii* antibodies; 88 (20.75%) were found seropositive for IgG, while 20 (4.72%) samples were seropositive for IgM. Regarding occupation, the highest percentage for chronic toxoplasmosis was reported in workers followed by policemen and pensioners at rates of 23.96%, 23.6%, and 23.07%, respectively. The age group 18-30 years showed the highest seropositive rate which was 26.6%; the seropositivity rate decreased with the increase of age. Regarding the blood groups, the highest percentage of IgG was reported in both O⁺ and B⁻ groups, which were 23.2% and 23.1%, respectively. The higher rate of seropositivity was observed among married males (21.32%) as compared to unmarried males (19.05%) and fertile (22.3%) versus sterile males (12.5%). Concerning IgM antibodies, the highest seropositivity (9.4%) was reported in infertile males. The results of this study showed the importance of demographic factors to the epidemiology of *T. gondii* in males, which support the role of public health in the control of infectious diseases.

Key words: Fertility, Male, Seroprevalence, Toxoplasmosis.

Introduction:

Toxoplasmosis is a significant zoonotic disease caused by an obligate intracellular protozoan parasite called *Toxoplasma gondii* (*T. gondii*), which causes infections in all warm-blooded animals (1). Toxoplasmosis has a worldwide distribution, and it's been estimated that *T. gondii* infects about one-third of the human population (2). Humans and other animals, including birds can serve as intermediate hosts, while felids like domestic and wild cats are considered as definitive hosts (3). There is a worldwide variation in the prevalence of toxoplasmosis, being higher in underdeveloped countries than developed ones (4). This variation in prevalence can be explained by several factors including the climate, number and presence of cats, age, nutritional and factors, and cultural and ethnic practices (5, 6). Direct contact with cats is not required for transmission due to the longevity of oocysts in the environment (5).

Scientific studies have reported that *T. gondii* infections in males affected reproductive parameters; the latest studies have concluded that transmission of toxoplasmosis can occur by sexual contact between couples (7). Furthermore, other studies have demonstrated that sexual transmission of *T. gondii* can cause complications such as infertility (8). The fertility of males can be affected by congenital or acquired urogenital abnormalities, urogenital tract infections, increased scrotal temperature, endocrine disturbances, genetic abnormalities, and immunological factors (9).

In the Duhok province, limited studies have been performed on the seroprevalence of toxoplasmosis. These studies concentrated on the prevalence of toxoplasmosis among females without considering its effects among males (10). Furthermore, they did not estimate the general epidemiological status and risk factors associated with the prevalence of this disease among males. However, recently Hussien *et al.* (11) studied the relationship between toxoplasmosis and male sex hormones. This study is an attempt to correlate the epidemiology of toxoplasmosis and various

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demographic factors among males using ELISA technique.

Materials and Methods

During the period from October 2016 to November 2017, a total of 424 blood samples were collected from males aged (18-60) years, which belong to different socioeconomic classes. From each person, 5ml of blood was withdrawn and transferred to a clean fully labeled tube, then centrifuged at 3000 rpm for 10 minutes to separate the serum. The separated serum was dispensed into two Eppendorf tubes (fully labeled with the required information according to the questionnaire sheet designed for the study) and stored at -20°C.

The seroprevalence of *Toxoplasma* antibodies was determined using the ELISA technique. The ELISA kits were supplied by Bioactive Company (Germany). The procedure was done

according to the instructions provided with kit. The work was performed at the Central Blood Bank Laboratory in Duhok province.

Statistical Analysis

The data was analyzed based on age group, occupation, marital status, blood groups, and fertility, using online Open Epi program: Open Source Epidemiologic Statistics for Public Health (12) Version 3.01 www.OpenEpi.com.

Results:

The overall seroprevalence rate of *T. gondii* among the 424 blood samples was 108(25.47%), of which 88(20.75%) were seropositive for IgG, and 20(4.27%) were seropositive for IgM; the remaining samples were negative as shown in Table 1.

Table 1. The seropositivity rate of anti-*Toxoplasma* IgG, IgM antibodies in males using ELISA technique.

No. of tested Samples	Total positive		IgG +		IgM +	
	No.	%	No.	%	No.	%
424	108	25.47	88	20.75	20	4.72

In terms of occupation, the highest percentage for IgG was reported in the worker's group followed by policemen and pensioners, which were 23.96%, 23.6%, and 23.07%, respectively.

While the highest percentage (9.09%) for IgM was reported in mechanical workers group, these differences were statistically non-significant ($p>0.05$) as indicated in Table 2.

Table 2. The percentage of seropositivity of anti- *T. gondii* IgG and IgM according to the occupation.

	Samples number	Total positive		IgG		IgM	
		No.	%	No.	%	No.	%
Employ	87	20	22.99	17	19.54	3	3.45
Students	46	6	13.04	5	10.87	1	2.17
Policemen	89	24	26.97	21	23.6	3	3.37
Pensioners	39	11	28.21	9	23.07	2	5.13
Mechanic	11	3	27.27	2	18.18	1	9.09
Drivers	19	4	21.05	3	15.79	1	5.26
Workers	96	31	32.29	23	23.96	8	8.33
Farmers	5	1	20.00	1	20.00	0	0.00
Butchers	32	8	25	7	21.88	1	3.13
Total	424	108	25.47	88	20.75	20	4.72

Chi-square= 11.73, p-value = 0.16

In terms of age, the highest seropositive percentage was observed among ages from (18-30) years, which was 26.6%, and then the seropositive rate decreased with the increase of age, as shown in

Table 3. These differences were statistically non-significant ($p>0.05$). While IgM antibodies showed the highest rate (8.6%) among the age group (41-50) years.

Table 3. The seropositivity rates of anti- *T. gondii* IgG and IgM Antibodies among different age groups.

Age Groups	Samples number	Positive samples		IgG		IgM	
		No.	%	No.	%	No.	%
18-30	120	35	29.17	32	26.7	3	2.5
31-40	174	43	24.71	35	20.1	8	4.6
41-50	93	24	25.81	16	17.2	8	8.6
51-60	37	6	16.22	5	13.5	1	2.7
Total	424	108	25.47	88	20.8	20	4.72
		Chi-square 5.52		p-value = 0.13			

With respect to blood groups, the highest percentage of IgG was reported among men with both O+ and B- groups, which were 23.2% and 23.1% respectively, followed by AB- which was

20%. This diminutive difference in results was statistically significant ($p < 0.05$) as shown in Table 4.

Table 4. The seropositivity rate of anti- *T. gondii* IgG and IgM antibodies among different blood groups.

Blood group	Samples no.	Positive samples		IgG		IgM	
		No.	%	No.	%	No.	%
A+	104	28	26.92	20	19.2	8	7.69
A-	11	2	18.18	2	18.2	0	0
B+	100	28	28	21	21	7	7
B-	13	4	30.77	3	23.1	1	7.69
O+	138	36	26.09	32	23.2	4	2.89
O-	14	2	14.29	2	14.3	0	0
AB+	39	7	17.95	7	17.9	0	0
AB-	5	1	20	1	20	0	0
Total	424	108	25.47	88	20.8	20	4.72
		Chi-square 22.47		p-value = 0.002			

Married men showed a slightly higher overall seropositivity rate as compared to unmarried (25.71% versus 24.76%), but this difference was statistically non-significant ($p > 0.05$). The seropositivity rate of IgG in married men was also slightly higher than that of unmarried men, but this

difference was also statistically non-significant ($p > 0.05$). On the other hand, unmarried men showed a higher IgM seropositivity rate as compared to married men, but the difference between both values was, again, statistically non-significant ($P > 0.05$) (Table 5).

Table 5. The percentage of seropositivity of anti-*T. gondii* IgG and IgM between married and unmarried men.

Marital status	Samples number	Positive samples		IgG		IgM	
		No.	%	No.	%	No.	%
Single	105	26	24.76	20	19.05	6	5.71
Married	319	82	25.71	68	21.32	14	4.39
Total	424	108	25.47	88	20.75	20	4.72
		Chi-square 0.28		p-value = 0.59			

Fertile men showed the highest percentage of seropositivity for *Toxoplasma* IgG antibodies (22.3%), which was statistically significant ($p < 0.01$). While the highest seropositivity of IgM

antibodies reported among infertile men was 9.4%, and this difference was statistically significant ($p < 0.05$) as indicated in Table 6.

Table 6. The percentage of seropositivity of *T. gondii* regarding fertility.

Fertility	Samples number	Positive samples		IgG		IgM	
		No.	%	No.	%	No.	%
Fertile	287	75	26.13	64	22.3	11	3.8
Infertile	32	7	21.88	4	12.5	3	9.4
Total	319	82	25.71	68	21.32	14	4.39
		Chi-square 4.80		p-value = 0.02			

Discussion:

The pattern of seropositive rates in males for acute and chronic toxoplasmosis in the present study agreed, to some extent, with the study of Hussein *et al.* (11) conducted in Duhok province. Which also reported slightly lower rates for total and chronic seroprevalence (22.98% and 16.35%) and a slightly higher rate (6.25%) for acute seroprevalence of toxoplasmosis. Similarly, a study in Kirkuk by Obaid *et al.* (13) also reported higher rates of total and chronic seroprevalence of toxoplasmosis about (18.6% and 11.6%), and a lower rate (4.6%) for acute toxoplasmosis. A study by Bahhaj R *et al.* (14) also reported a high seropositive rate for IgG (39.36%) and a low seropositive rate for IgM (1.06%) among healthy blood donors.

The seroprevalence rate of toxoplasmosis can be affected by many factors such as the nature of the work, the type and source of food and drinks, and the sanitary conditions of food handlers (10). These results disagreed with a study performed by Al-Bajalan *et al.* (15) in which the highest seroprevalence percentage was reported in the mechanic group followed by the farmer group which were 71.42% and 63.63%, respectively. Also, using ELISA technique, Sarmad (16) reported a high rate (22.73%) of toxoplasmosis among farmers living in Erbil province and attributed it to their direct contact with soil contaminated with sporulated oocysts.

Regarding age, the highest seropositive percentage was observed among ages ranging from 18-30 years, which was 26.6%, and then the seropositive rate decreased significantly with the increasing age. Similarly in two studies performed in Baghdad, the first by Mahmood *et al.* (6) they reported high positive rates (24.79%) of IgG, using ELISA, at ages ranging from 18-25 and 26-33 years, which decreased with the increase in the age. Muhsin *et al.* (17) also reported the highest rate of toxoplasmosis in males was at ages between 19 -28 years and the lowest rate at the ages of 49- 58 years, which were 35.29% and 12.94%, respectively. In the present study, IgM antibodies showed the highest rate (8.6%) among the age group (41-50) years, while the Muhsin *et al.* (17) study reported the highest rate was among young ages (18-28) years and attributed it to higher activities and more significant exposure to risk factors as compared to older ages.

With respect to blood groups, the highest percentage of IgG was reported among men with both O⁺ and B⁻ groups, which were 23.2% and 23.1%, respectively, followed by AB⁻ (20%). These results agree with the study of Obaid *et al.* (13) who

reported the highest percentage of IgG antibodies among blood donors in group A⁺ was 33.3% followed by O⁺ and AB⁻ groups 19.2% and 16.6% respectively, whereas disagreed with B⁻ group which was 0%. Moreover, in other studies in Baghdad, the highest seropositivity rates of toxoplasmosis were reported among men belonging to other blood groups such as Mahmood *et al.* (6) who showed that the highest prevalence was among men having AB⁺ (41.32%) and the lowest prevalence (15.70%) of group O⁺. While Abdulla *et al.* (18) reported the highest rate (26.1%) among blood group O⁺, they attributed this difference in the results to several factors such as the possibility of the molecular mutation of strains among patients.

Married men showed a slightly higher overall seropositivity rate as compared to unmarried men (25.71% versus 24.76%). The seropositivity rate of IgG in married men was also slightly higher than that of unmarried men, but the differences in seropositivity among both groups were statistically non-significant ($p > 0.05$). Moreover, unmarried men showed a higher but non-significant ($p > 0.05$) IgM seropositivity rate as compared to married men.

The present results conflict with those of Al-Aboody (19) in Thi-Qar who stated that chronic and acute toxoplasmosis infection in married males was significantly higher than unmarried ones, which was 55 (78.5%), 4 (5.7%) and 10 (14.2%), 1 (1.4%) respectively. Similarly, Muhsin *et al.* (17) in Baghdad, reported a significantly higher rate of chronic toxoplasmosis infection among married men as compared to unmarried men, which were, 70 (82.35%) and 15 (17, 64%) respectively. In Duhok province, there are no previous studies on males infected with toxoplasmosis to compare the obtained results.

Fertile men showed the highest percentage of seropositivity for *Toxoplasma* IgG antibodies (22.3%), which was statistically significant ($p < 0.01$) while the highest seropositivity of IgM was reported among infertile men (9.4%), which was significantly ($p < 0.1$) different from unmarried. There is little information about the involvement of *T. gondii* with male infertility. Zhou *et al.* (20) reported a higher rate of toxoplasmosis in infertile human couples than infertile ones, and related it to the anti-sperm antibodies which were higher in infected couples. Also, Qi *et al.* (21) studied 100 cases of male sterility and found that 36% were seropositive for *Toxoplasma* IgG and IgM.

Dongmei *et al.* (22) stated that apoptosis of spermatogenic cells happens in fertile and infertile males, and they found a very close relationship between sperm apoptosis and male infertility,

suggesting that *Toxoplasma* infection may cause the apoptosis of spermatogenic cells.

The present results disagree with Mahmood *et al.* (6) in Baghdad, who reported a higher seropositivity rate (69.30%) of IgG in infertile men as compared with fertile men (30.69%). Males infected with acute toxoplasmosis had the highest percentage of anti-*Toxoplasma* IgM antibodies (85.71%) as compared to infertile (14.2%). In contrast, different results achieved by Al-Aboody (19), in Thi-Qar, showed that fertile males had a low percentage of anti-*Toxoplasma* IgG antibodies while infertile males showed a high percentage about 18 (30.5%) and 37(62.7%) respectively.

Different results can be obtained within the same country; this may be due to the contribution of different factors such as the source of serological test kits (which vary with manufactured companies), the study and laboratory conditions. Furthermore, nutritional behaviors vary within the same country, as well as hygienic habits, climate conditions, having pets, working or cleaning gardens, etc (23).

Conclusion:

The present study can be considered as an initial step in Duhok province to investigate the prevalence of toxoplasmosis among males from different ages, socioeconomic classes, and relation to demographic factors. Based on the outcomes of this study, we can conclude that males, much like females, can get toxoplasmosis infections. Moreover, toxoplasmosis still shows a significant threat to both genders. The current results indicate that toxoplasmosis has a high prevalence among males, especially workers who may practice poor hygienic habits and have low levels of health education, in addition to the abundance of stray cats in the community, which can contaminate the environment with the oocysts. Therefore, efforts must be made to achieve a reasonable control on food safety, especially among food handlers, and on stray cats, in order to reduce the risks of infection with toxoplasmosis.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.

- Ethical Clearance: The project was approved by the local ethical committee in University of Duhok.

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الانتشار المصلي وبعض العوامل الديموغرافية المرتبطة بعدوى المقوسات الكوندية بين السكان الذكور في محافظة دهوك / العراق

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الخلاصة:

هدفت الدراسة الحالية إلى دراسة معدل الانتشار المصلي لعدوى المقوسات الكوندية وعلاقته ببعض العوامل الديموغرافية بين الذكور في محافظة دهوك/ العراق. تم جمع ما مجموعه 424 عينة دم عشوائية من السكان الذكور من مختلف الأعمار (18-60) سنة وفئات اقتصادية اجتماعية مختلفة. من بين 424 عينة تم فحصها، كانت 108 (25.47%) إيجابية المصل منها 88 (20.75%) تم العثور عليها إيجابية مصليا ل IgG، في حين أن 20 (4.72%) عينة كانت إيجابية مصليا ل IgM. فيما يتعلق بمهنة الذكور المأخوذة منهم العينات، أعلى نسبة لداء المقوسات المزمّن اثبتت مصليا عند العمال، يليهم رجال الشرطة والمتقاعدون بمعدلات 23.96% و 23.6% و 23.07% على التوالي. أظهرت الفئة العمرية 18-30 سنة أعلى معدل مصل الدم الذي كان 26.6%. مما يعني انخفاض معدل الإيجابية المصلية مع زيادة العمر. فيما يتعلق بفصائل الدم، كانت أعلى نسبة مصليا ل IgG في كل من فصيلة الدم O + و B-، والتي كانت 23.2% و 23.1% على التوالي. وقد لوحظ ارتفاع معدل إيجابية المصل لدى الذكور المتزوجين (21.32%) بالمقارنة مع الذكور غير المتزوجين (19.05%) والخصبة 22.3% مقابل الذكور العقيمة 12.5%. تظهر هذه الدراسة أهمية العوامل الديموغرافية لوبائية المقوسات الكوندية لدى الذكور، والتي تدعم دور الصحة العامة في السيطرة على الأمراض المعدية.

الكلمات المفتاحية: الخصوبة، الذكور، المصلي، داء المقوسات.