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Occurrence of Toxoplasmosis in Baquba City, Diyala, Iraq

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ABSTRACT

The current study was conducted to determine the occurrence of toxoplasmosis in patients presented to Emergency Department, General, Educational Baquba Hospital, and Al-Batool Hospital in Baquba City, Diyala Province, Iraq, on the dependence of Serological Examinations from November 2018 to February 2019.

Blood samples were collected from, 30 young females, 218 adult women, 49 young males and 203 adult men, Total, 500, suffered from different disease conditions (pregnancy troubles, hormonal disturbances, diabetes, healthy etc..). Latex Agglutination Test (LAT), Enzyme Linked Immunosorbent Assay (ELISA), were used to evaluate the presence of anti-Toxoplasma antibodies.

The results showed that, the percentage of positive reactors recorded by LAT test were 205 /500; 41 %, of which, 99\500; 19.8% in adult women, 7\500; 1.4% in young women, 81\500; 16.2% in adult men and 18\500; 3.6% in young men. While in ELISA, 22\100 ; 22%. Titer level of anti-*Toxoplasma gondii* antibodies ranged between (1/2 to 1/128). The infections were higher in adult women and men than young persons. The infections were higher in women than men.

In conclusion, titer levels referred that the patient suffer from chronic or carrier state.

Introduction

Toxoplasmosis is result from infection with, *Toxoplasma gondii*, a ubiquitous obligate intracellular pathogenic protozoan, widely prevalent in humans and animals [1- 4]. An important zoonotic parasitic disease that affects millions of people, has a worldwide distribution in human populations infecting up to one third of the global population and a wide range of mammalian and avian species [5- 7]. Present in hot, humid countries, able to develop in a wide variety of vertebrate hosts. Cats and other members of Felidae are definitive hosts, while human and wide range of animals, birds and rodents act as intermediate hosts [8].

In immune competent individuals, preferentially infects tissues of central nervous system, be a contributing factor to certain psychiatric disorders [9 , 10].

The Seroprevalence of Toxoplasmosis estimated for human population varies greatly among, different countries, different geographical areas, within one country, even within a same city [11,12]. The various prevalence of the disease may be associated with the

geographical location and type of serological tests used, as these serological tests vary in their sensitivity and specificity to toxoplasma antibodies [13].

Diagnosis of Toxoplasmosis infection is seldom made by recovery of the parasite, usually done by serological tests [14] or histocytologic examination, but serologic test such as Latex Agglutination Test(LAT), and Enzyme Linked Immunosorbent Assay (ELISA) for antibodies detection has been more full and adequate tool to diagnose toxoplasma infection in both human and animals [15,16].

A variety of serological tests for *T. gondii* antibodies have been used as an aid in diagnosis of acute infection and to assess previous exposure to the organism. Use of serological tests to show specific antibodies to *T. gondii* is the primary method of diagnosis. The problem with serologic diagnosis is that antibodies to *T. gondii* is present in relatively high numbers of individuals in most populations. These antibodies titers may persists at high levels for years in healthy people [17].

The aims of present study was, determine the prevalence of *toxoplasma* infection, according to sex, age through examination of anti – toxoplasma antibodies in serum of peoples in Baquba City, Province of Diyala, Iraq.

Materials and Methods

The study was conducted from November 2018 till February 2019. Blood samples were collected from patients presented to Emergency Department, General, Educational Baquba Hospital and Al- Batool Hospital in Baquba City, Province Diyala, Iraq.

Five hundred 500 serum samples were collected, represented 218 adult women suffered from hormonal and pregnancy disturbances; 30 young women, 49 young men, and 203 mature men healthy or suffered from general disease conditions, or accidental cases. The sera were submitted for LAT and ELISA tests (Table 1)

LAT test in which, Antigen coated polystyrene latex particles were used, suitable for screening large numbers of samples [18- 20]*. In ELISA, soluble antigen is coated to micro titer plates and serum is added to form an antigen- antibody complex (if specific antibodies are present) a secondary enzyme –

linked antibody specific to the host species is added to detect antigen- antibody complex** [21].

* [Haansberg, Netherlands, Salucea, Toxo LATEX KIT)

**Chekit-Toxo Test-Switzerland

Data analysis

Data were analyzed Chi-square and P<0.05 was considered to be significant [22].

Results

Latex Agglutination Test (LAT):

Total positive samples for *T. gondii* antibodies were 205\ 500; 41%, from which 7 \ 500; 1.4% represented young women, 99\500;19.8% adult women, 81 \500; 16.2% adult men, and 18\500; 3.6% young men (Table 1).

Table (1) represent results of LAT test:

Sex	Numbers	positive	%
adult women	218	99BC	45.4
young women	30	7A	23.33
adult men	203	81BC	39.9
young men	49	18B	36.7
Total numbers	500	205	41

A,B,C, significantly different in comparison between groups (age, Sex) at P<0.05 level

Table (2) represent results of LAT test:

Age\ sex	AL-Batool Hospital			Emerg. Depart. Gener. Hospital			Both hospitals		
	Total No.	+ve	%	Total No.	+ve	%	Total No.	+ve	%
Adult women	108	49BC	45.4B	110	50BC	45.5B	218	99BC	45.4B
Young women	15	4A	26.7A	15	3A	20A	30	7A	23.3A
Adult men	27	12B	52.2B	176	69BC	39.2B	203	81BC	39.9A
Young men	23	7A	25.9A	26	11B	42.3B	49	18B	36.7A
Total	173	72a	41.6	327	117a	35.8	500	205b	41

A,B,C, significantly different in comparison between groups (age, sex), a, b, between (hospital) at P<0.05 level

ELISA test

The result of current study showed that among a total of 100 samples, 22) samples were react positively to ELISA, from which 2\100; 2% represent young women, 12\100; 12% adult women, 1\100; 1% young men, and 7\100; 7% adult men (Table 3)

Table (3) Represent results of ELISA test

Patient	Age	No. of +ve	% of +ve	No. of -ve
Women	Young	2	2 A	12
	Adult	12	12 BC	43
Men	Young	1	1 A	2
	Adult	7	7 B	21
Total		22	22	78

A,B,C, significantly different in comparison between groups (age, sex) at P<0.05 level

Level (Titer) of Anti *T. gondii* antibodies :

In LAT test the serum dilution which gave positive reaction to *T. gondii* ranged between 1\2 to 1\ 128 . Level 1\2 were highest in number 113\205; 55.1%, Highest was in adult women 59\ 205; 28.8%, the lowest was 3\205; 1.5% in young women, while in adult men were 41\205; 20%, and 10\205; 4.9% in young men. Followed by 1\4 as it was 40\ 205; 19.5%, then 1\8 ; 22\205 ; 120.7% , while 1\16 was 14\2305; 6.8% , meanwhile 1\32 was 8\205; 3.9% , each of 1\64 and 1\128 was 4\205; 1.95% (Table 4).

Table (4) Titer of anti *T. gondii* antibodies.

Age\ sex	Total	Titer						
		1\2	1\4	1\8	1\16	1\32	1\64	1\128
Adult women	99	59BC	20B	7B	6B	3B	2A	2A
Young women	7	3A	2A	1A	1A			
Adult men	81	41BC	15B	10BC	7B	4B	2A	2A
Young men	18	10B	3A	4B	-	1A		
Total	205	113bcd	40bc	22bc	14b	8b	4a	4a

A,B,C, significantly different in comparison between groups (age, sex), a, b, c, d between level of titer at P<0.05 level

Relationship between age groups and percentage of infection:

Results revealed that the highest figure in positive reactions were in age group (16-20), 55\205; 26.8% ,

followed by (31-35) 42\ 205; 20.5%, and the lowest numbers were in age groups (56-70) 2\205; 0.97% (Table -5-).

Table (5) relationships between age groups and percentage of infections

Sex	No. and %	Total	Age groups									
			1-12	16-20	21-25	26-30	31-35	36-40	41-45	46-50	51-55	56-70
women	Total No.	248	30	59	28	24	36	24	19	7	14	7
	+ve	104	5A	24A	14A	12A	24A	12B	7A	2A	3A	1
Men	Total No.	252	39	71	28	36	38	14	14	5	5	2
	+ve	101	7A	31A	12A	16A	18A	6A	6A	2A	2A	1
	Total +ve	205	12bc	55bcd	26bc	28bcd	42bcd	18bc	13bc	4b	5b	2a

A,B,C, significantly different in comparison between groups (sex), a, b, c, d between age groups, at P<0.05 level

Time of Positive Reaction in LAT Test

Results of study revealed that the highest numbers of positive reactions were within (2 and 3) minutes post mixing Ag and Ab. and the lowest were within (5) minutes of mixing Ag with Ab. (Table 6).

Table (6) numbers of samples react positively with time of appearance of reaction

Age \ Sex	Times : minutes				
	1	2	3	4	5
	No. of samples				
Adult women	27B	66B	65B	55BC	5A
Young women	1A	2A	2A	1A	
Adult men	16B	74B	74B	37BC	10B
Young men	1A	2A	2A	3B	
Total	45b	144bc	143bc	96bc	15a

A,B,C, significantly different in comparison between groups (age, sex), a, b, c between times of reaction at P<0.05 level

Discussion

LAT test:

Out of total 500 serum samples in this study, 205 showed a seropositive reaction for toxoplasmosis, giving an incidence rate of 41%.

T. gondii infection in humans is widespread throughout the world. Approximately half billion humans have antibodies to *T. gondii*. The incidence of infection in humans and animals may vary in different parts of a country. The cause of these variations may be due to environmental conditions, cultural habits, and animal species are among factors that may determine the degree of natural spread of *T. gondii* [23]. Approximately one-thirds of all humanity has been exposed to this parasite, but the seroprevalence varies considerably between countries and population group [12].

Socioeconomic and environmental factors have been associated with transmission and consequently, a higher prevalence of *T. gondii*, for example, intake of raw or undercooked meat containing tissue cysts, contact with oocysts- contaminated food, water, and soil, and mother to offspring transmission, are some factors involved in the epidemiology of this disease [24-30].

Humans may remain infected for life and will stay asymptomatic unless immunosuppression occurs [31].

Since people in Iraq do not have the habit of consuming raw meat, it is highly probable that accidental ingestion of oocysts is the primary route of transmission in Iraq.

In Iraq, Ehsan, [32] referred that of pregnant women (suspected cases) LAT test showed infection rate of 32.43%. [33] found that 57\68 ; 83.82% of women were react positively to LAT test in Baquba City, Diyala, Iraq.

In many Iraqi Provinces, similar studies were done, [34] reported low rate of (8.6%) positively from eight Governorates in Iraq. While [35], reported a prevalence rate of (20.4%) toxoplasmosis among Iraqi women. Study by [36] showed a rate of 18.5% of toxoplasmosis antibodies in Basarah population. Meanwhile [37] found the infection in Baghdadi women to be 19.17%, while [38] recorded 26.8% in Al-Najaf Province., [39] 22% in blood donors. [40] recorded 29.25% in Baghdad. [41] 46.65% in



Figure 1- Showing reaction plate of LAT test

Diwanyia. [42] 45.8% in women in Kut City. [43] found that the prevalence of toxoplasmosis antibodies among women in Baghdad was 39%.

Yacoub *et al.*, [44] found that the prevalence of toxoplasmosis in Basrah had been shown to be 41.1 to 52.1% . [45] showed the presence of infection around 49.95% in Tikrit.

Razzak *et al.*, [46] found that from 187 of the examined women by LAT test, 55 gave positive reaction. High seropositive has, however, been reported in this region of Iraq. [47] studied 320 persons in Duhok Province and found that 134 were positive by LAT. [48] reported a seropositive of 39.33% by LAT in Mosul .

Globally the incidence of toxoplasmosis varies in different countries. In Czech Republic the rate of toxoplasmosis in blood donors tested scored 34.1 and 27.1% in men and women respectively [49].

Relationship of occurrence with age and sex

In current study, there was a significant relationships of age and sex with occurrence of toxoplasmosis in Baquba, as the infection was of the highest level in adult women and men in comparison with young women and men. As the infection rate in adult women were 99\500; 19.8%, followed by adult men 81\ 500, 16.2%, and young men 18\500;3.6% and the lowest was in young women 7\500; 1.4%.

Mohanad *et al.*, [50] showed that, a decreasing seroprevalence was observed in pregnant women, recorded that from 91 couples examined for antibodies of *T. gondii* using ELISA in Ramadi City, the overall anti *T. gondii* IgM and IgG in both couples were 38.4%. The seroprevalence in wives was only 30.7%, while in husbands was 13.1%. Abortive women and abnormal pregnancy had the highest percentage rates 35.7 versus 57.14% of toxoplasmosis among those of (25 to 30) years old and the lowest was among those who have the average of age (35 to 40) years old.

Al-Musaway,[51] referred that out of 319; 111 males and 208 females of (18-42) years old in Thi-Qar using ELISA test 21.94% were react positively, and there were significant differences between male and females 7.52% in males and 14.42% in females who carried the anti- toxoplasma antibodies.

The results of the current study revealed that the highest figure in positive reactions were in age group (16-20) 55\205; 26.8% , followed by (31-35) , 42\ 205; 20.5%, and the lowest figures was in age groups (56-70) 2\205; 0.97%. This can attributed to the numbers of peoples examined.

Al- Saady, [52] found that among men in Baghdad of (18-25) years old by using LAT and ELISA tests in searching for IgM and IgG, 136\ 400 (34% react positively to LAT and (121\ 400; 30.25% react positively to IgG in ELSA. and only 10 men showed positive reaction to IgM in ELISA. There were significant differences between age groups as (18-25) and (26-32) year the highest percentage and (50-57) was the lowest. However, [53] showed that male

more susceptible than female to many seropositive parasites

Al-Ghezy [54] referred that the highest infection rate has scored in age group (36-40) years 30.5%. In the examining of 400 serum samples of aborted women by using ELISA test, 92 samples 23% and the highest infection rate has scored in age group (36-40) years 30.5%.

In a study of prevalence of toxoplasmosis in pregnant women in Al-Muthana Province – Iraq, [55] found that there was significant effect of age on proportion rate which increase directly with age, highest infection rates were in (35-39) age group, while lowest at (15-19) age group. The increase in the level of toxoplasmosis infection with increasing age was also confirmed by other studies.

Jassam, [56] recorded that the age group of (20-29) years had the lowest rate 28.6% and the age group of (30-39 ; 40-49) and (≥ 50) with the highest rates 48.6% , 44% and 58.1% respectively.

Several results of different regions that indicated the percentage of anti *T. gondii* antibodies was increased with age (57), with only a few studiers failing to identify such an association (58-60).

The overall seroprevalence of toxoplasmosis in South Africa was 18.15%, while among males was 16.7% and females 18.6%. The serologic evidence of toxoplasmosis in Ethiopia was found in 60% of them. The overall anti *T. gondii* IgG prevalence in China was 12.3% [10].

Williams *et al.*, [61] pointed that the incidence increases with age but to a peak of 34 years . In Iran [62] showed that the seroprevalence of toxoplasmosis was 48.8% in men and 55.2% in women. Other results showed no significant difference with age factor but the highest infection rate occurred in age group (31-35) years [63].

High prevalence values of infection with *T. gondii* were found in adult women, this probably happened due to more frequent exposure to toxoplasma through cat's contact, soil exposure [64]. The difference of results between studies can attributed to the differences in the specificity and sensitivity of methods used for examining the response against the parasite [65].

This rising trend with age, reflects the continuity of risk infection throughout adult life and arises from the accumulative risk of exposure and infection with age in an environment where transmission is encouraged by the high density of feral cats [66].

Level of titer of anti- toxoplasma antibodies

In current study, the titer level of anti- toxoplasmosis antibodies ranged between 1\2 to 1\128, with a significant differences at ($P > 0.05$) in relation with different age groups. Higher number were in 1\2 titer level; 113\205; 55.1%, followed by 1\4; 40\205; 19.5% then 1\8; 22\205; 10.7%) , 1\16 ;14\205; 6.8% , 1\32; 8\205; 3.9% and each of 1\64 and 1\128; 4\205; 1.95%.

Most of the samples examined by LAT had low titration 1\10- 1\40 in both married and none married women [67]. [11] showed that the results of antibodies titer by LAT was 1\128 which is reported in 25.64%.

Ehsan [32] showed that titer was ranged between 1\4 to 1\32 which indicated chronic infection. It had been concluded that toxoplasmosis were important infectious diseases affected both women and animals in Ninevah Province.

In City Santaren in the State of Para between (1977-1999) recorded a distribution of 72.72% of toxoplasmosis in 601 of human: 41 of them was pregnant women , and the prevalence was 82.9% of antibodies [68].

These variables results can attribute to the differences in the samples used in each study and their variables conditions and data of studies, the geographical

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location and the type of serological tests[13]. As the serological tests varies in their sensitivity and specificity to toxoplasma antibodies [33] found that LAT test detect more cases react positively to toxoplasma, while ELISA came second to it and Casset test the third one, even it did not gave any reactions to toxoplasma.

In conclusions: the results revealed the fact that adult women showed the highest infection rates. The antibodies titers ranged between 1\2-1\128 with the most cases showed 1\2 level which indicated that most of tested patients are of chronic or subclinical state of infection. Further studies can be done to determine the genotyping of the parasite, as there are variations in virulence, which mean that there are different genotype of parasites present in different geographical areas in the world.

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حدوثية داء المقوسات Toxoplasmosis في مدينة بعقوبة / ديالى / العراق

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الملخص

انجزت الدراسة الحالية لتحديد انتشار الاصابة بطفيلي داء المقوسات في المرضى المراجعين لقسم الطوارئ - المستشفى العام التعليمي، ومستشفى البتول للولادة والاطفال، بعقوبة، محافظة ديالى، العراق، باعتماد الفحص المصلي من تشرين الاول 2018 الى شباط 2019. جمعت نماذج من الدم من 30 انثى يافعة، 218 انثى بالغة (248 انثى) ، و 49 ذكر يافع، 203 ذكر بالغ (252 ذكر) ، وبذلك يكون العدد الكلي 500 نموذج دم. والذين كانوا يعانون من حالات مرضية مختلفة كاضطراب في الحمل، الاختلال الهرموني، داء السكري ، الخ.. اعتمد اختبار لاتكس التلازني (LAT) Latex agglutination test، والاليزا (ELISA) لتقييم وجود اضرار المقوسات . اشارت النتائج الى ان التفاعل الموجب الكلي المسجل في اختبار لاتكس 205 \ 500؛ 41%، منها 99 \ 500؛ 19.8% في الاناث البالغات، 500 \ 7؛ 1.4% في الاناث اليافعات ، 81 \ 500؛ 16.2% في الذكور البالغين، 18 \ 500؛ 3.6% في الذكور اليافعين. اما نتائج اختبار الاليزا فكانت 22 \ 100؛ 22% . تراوح معيار اضرار المقوسات بين (1 \ 2 الى 1 \ 128) . سجلت الدراسة ان الاصابة بداء المقوسات ، كانت الاعلى في الاناث والذكور البالغين، والاكثر في الاناث مما في الذكور. ويشير مستوى الأضرار الى ان الاشخاص قيد الدراسة اما حاملتي الطفيلي او مصابين باصابة مزمنة.