



Original Research Article

# Utilization of Molecular and Serological Methods to Investigation *Toxoplasma gondii* in Healthy Apparently Students in Babylon Province

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#### Abstract

Toxoplasmosis, an infection caused by *Toxoplasma gondii*, is generally asymptomatic or is associated with mild, non-specific clinical manifestations in immunocompetent patients. The present study consider the first trial in Babylon province to detection of toxoplasmosis among healthy university and secondary students, from February to May 2015, collect 112 blood sample from healthy apparently students. The results of serological test revealed that 57/112 (50.8%) was positive for *T. gondii* in LAT test, (25%) for university students and (25.8%) for secondary students, there is a significant difference ( $p \le 0.05$ ) between male (27.6%) and female (23.2%) in toxoplasmosis infection, where as 21.4% was positive for ELISA in which 13.8% positive for IgG antibodies and 8.0% positive for IgM, while nPCR results showed 19 out of 112(16.9%) was positive to toxoplasmosis. The presence of *T. gondii* antibodies in the serum regarded as the important criterion for the diagnosis of toxoplasmosis but these methods insufficient to diagnosis, it must be combine with molecular methods such as polymerase chain reaction which detect *T. gondii* DNA in five or ten parasites are present in blood.

Key word: Toxoplasma gondii, nested PCR, ELISA, latex test.

استخدام الطرق الجزيئية والمصلية للكشف عن المقوسات الكوندية في الطلاب الاصحاء في محافظة بابل

#### الخلاصة

٨٠١٠٨ موجبة ل١٩٥ و ٥٨٨٠ موجبة ل١٩١٧ بينما اظهرت تناتيج ١١٣٥٢ أن ٢٦ عينة من أصل ٢١٦ بسبة ٢٠١٩ كانت موجبة لداء المقوسات. ان وجود الاجسام المضادة لداء المقوسات في المصل يعد معيارا هاما في تشخيص داء المقوسات لكن هذه الطريقة غير كافية في التشخيص لذا يجب ان تصاحب بالطرق الجزيئية مثل تقنية تفاعل السلسلة المتعدد التي تكشف عن دنا الطفيلي حتى في حالة وجود خمس او عشرة طفيليات في الدم.

الكلمات المفتاحية: المقوسات الكوندية ، تفاعل سلسلة البلمرة المتداخل، الاليزا، فحص التلازن.

# **Introduction**

oxoplasmosis is a disease that results from infection with the Toxoplasma gondii parasite. one of the most common parasite in word. It may cause flu-like symptoms in some people, but most people affected never develop sign and symptoms [1]. Toxoplasmsis in generally transmitted through oral route (ingestion of raw or undercooked meat, or product. meat-derived ingestion of unwashed fruits or vegetable or ingestion of contaminated ca feces) or by accidentally ingesting Toxoplasma gondii cysts. Also Toxoplasmosis can be transmitted congenitally from mother to fetus through the placenta[2].

*T. gondii* has a wide variety of hosts, as almost all warm blooded animals can be infected. Sexual replication of the parasite occurs only in domestic cats and wild felidae (definite hosts), while asexual replication occurs in both intermediate and final hosts. Oocysts are passed in the feces of cats and become infectious within 21 days of being shed. Tachyzoites survive and multiply only in an intra -cellular location while tissue cysts containing few or many bradyzoites occur in the tissues of infected animals within a week of infection [1].

Diagnosis of *T. gondii* can be made by direct observation of the parasite in stained tissue sections, cerebrospinal fluid or other biopsy material, however these techniques are used less frequently because of the difficulty of obtaining these specimens, also parasite can be isolated from blood or other body fluids but this process can be difficult and require considerable time[3].

Use serelogical of test for demonstration of specific antibody to T. gondii is the initial and primary method of diagnosis. Different serologic test often measure different antibodies that possess unique patterns of rise and fall with time after infection [4]. Serodiagnosis of acute toxoplasmosis is

based on the demonstration of significant increase in specific IgG or IgM antibodies level, however the prevalence of high T. gondii IgG antibody titer among individuals in most population and the sustained persistence of specific IgM antibodies peoples in some have complicated the interpretation of serological tests when acute toxoplasmosis is suspected. Many studies have shown high prevalence of T.gondii antibodies in healthy voluntary blood donors in urban Karnataka [5]. [6] used the titer of IgG and IgM antibodies as a test for diagnosis of T. gondii among couples. while [7]showed higher prevalence of Toxoplasmosis among health voluntary blood donors when used Elisa test for IgG and IgM antibodies. Also serelogical test such as latex agglutination test and Elisa were used for detection of T. gondii antibodies in meats [8].

In recent studies used molecular methods based on polymerase chian reaction (PCR)for detection of Toxoplasmosis. these methods are simple, sensitive, reproducible and can be applied to all clinical sample[9]. These methods based on detection of a specific DNA sequence of B1 gene using different assay and protocol such as conventional PCR, nested PCR and real-time PCR. Many studies used B1gene as a target gene, [10] concluded that nested PCR assay in blood has advantage in detection of recent and active toxoplasmosis. While [11] confirm that real-time PCR was a rapid, sensitive and quantitative methods for detection of T. gondii DNA in blood.

This study aimed to investigation the possible presence of *Toxoplasma gondi* in healthy apparently students by using serological and molecular methods.

# Materials and Methods

A total of 112 blood sample were randomly collected from apparently healthy voluntaries (male and female) from February - May 2015, for detection of Toxoplasmosis in healthy student in Babylon province. Voluntaries were divided into two groups, university student and secondary student. Five ml of venous blood was collected for using in serological and genetic detection of *Toxoplasma gondii*as following:

### 1-Serological diagnosis

A-Latex agglutination test (LAT):

The latex agglutination test (HumatexToxo) was used as screening test for serological demonstration of *T*. *gondii* according to manufactures instruction.

B-Enzyme linked immunosorbent assay (ELISA):

This test was done by using standard commercial kit (Biokit diagnostic

company, Spain) specific to *T. gondii* IgG and IgM antibodies according to manufactures instruction.

### 2-Genetic diagnosis

### A-DNA extraction:

DNA was extracted from whole blood by using DNA extraction kit (Bioneer, korea) according to manufactures instruction.

B- detection of *T. gondii* DNA by nested PCR assay :

nested PCR assay was used for amplification targeting B1 gene for *T. gondii* by using set of primers (580bp and 531bp) depended on [12].

Table 1: Nucleotides sequences of	f B1	gene primers	
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Primers		DNA sequence	Molecular weight
Outer primer	F	5 <sup>-</sup> TGT TCT GTC CTA TCG CAA CG-3 <sup>-</sup>	580bp
	R	5 <sup>-</sup> ACG GAT GCA GTT CCT TTC TG-3 <sup>-</sup>	
Inner primer	F	5 <sup>-</sup> -TCT TCC CAG ACG TGG ATT TC-3 <sup>-</sup>	531bp
	R	5 <sup>-</sup> -CTC GAC AAT ACG CTG CTT GA-3 <sup>-</sup>	

The procedure of nPCR consist of two round:

**A-** First round:

In this round we amplification of 580bp fragment of B1 gene, the final reaction volume equal to 20  $\mu$ l which consist of 10

 $\mu$ l of PCR premix, 2  $\mu$ l of outer primer (1 $\mu$ l for forward primer and 1 $\mu$ l for reverse primer), 1 $\mu$ l DNA template and 7  $\mu$ l of PCR water. Then the mixture was transformed into thermocycler as in table (2).

Table 2: Cycling condition of the first amplification

Step	Temp.	Time	No. of cycles
Initial denaturation	94	2 min.	1
Denaturation	93	10 sec.	40
Annealing	57	10 sec.	40
Extension	72	30 sec.	40
Final extension	72	5 min.	1

### **B**-Second round:

The fragment 531bp of B1 gene was amplificated in this round by using 10  $\mu$ l of first round product as template with adding Inner primer, then mixture transformed into thermocycler and programmed as in table (2) but used 65c° instead of 57c° in annealing step. all PCR product were electrophoresis on 1.5% agarose and examined under UV light [13].

### **Statistical Analysis**

All data were analyzed by SPSS software (version13) with fisher-exact test to evaluate the difference between the study groups, p value  $\leq 0.05$  was considered significant.

#### **Results and Discussion**

The results of our experience by using serological (LAT and ELISA) and nested PCR test for the identification of *T. gondii* in blood of healthy apparently students showed that 57/112 (50.8%) were positive to *T. gondii* by using LAT test, this results

agree with [8], over all the (112) healthy students voluntaries the percentage of infection in male was (10.7%) while in female (14.3%) in university students. Were as secondary students revealed (16.9%) in male and (8.9%) in female (table 3).

Table3: distribution of <i>T. gon</i>	<i>dii</i> infection ac	ccording to gender
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Gender	Total No.	infected No.	University	Secondary
			students (%)	students(%)
Male	54	31	12 (10.7)	19 (16.9)
Female	58	26	16 (14.3)	10 (8.9)
Total	112	57 (50.8)	28 (25)	29 (25.8)

The infection of male was significantly higher ( $p \le 0.05$ ) than female (31/54 for male and 26/58 for female) but there are no significant different ( $p \ge 0.05$ ) between university and secondary students . This result was in agree with [14] which showed that male in puberty age are more female susceptible than to get Toxoplasmosis infection or may be some of these infection occur in earlier age. study of [15] confirm that male is more susceptible than female to many types of parasites, sex differences in exposure as well as susceptibility to parasites probably contribute to sex-based differences in the intensity and prevalence of parasite, males are more likely to engage in behaviors such as aggression, dispersal and grouping that increase the likelihood of contact with parasites. The prevalence of toxoplasmosis in healthy apparently students may be associated with exposure to risk factors, such as contact with contaminated

soil, eat contaminated meat or vegetable, or unhygienic food preparation. Early study reported that *T. gondii*tachyzoites may be isolated from raw chicken egg laid by hens with experimentally induced infection [16]. Also animal meat may sever as potential source of infection for students, this results confirm by study of [12] who proved the presence of Toxoplasma B1gene in blood, liver, heart, pectoral muscles and small intestine of avian (chicken, turkey, geese and ducks) in middle Euphrates region.

We screened 112 blood sample by using serological and genetic tests, table (4) showed that 57/112 sample were positive for LAT, 24/112 were positive for ELISA while nPCR results showed 19/112 were positive in our study we used different methods to increase the accuracy of Toxoplasomsis diagnosis in healthy students.

Test	Positive (%)	Negative (%)
LAT	57 (50.8)	55 (49.2)
ELISA	24 (21.4)	88 (78.5)
nPCR	19 (16.9)	93 (83.0)

Table 4: distribution of T. gondii infection according to diagnostic diagnostic technique

The results of our study was in agree with results of [17] who found that percentage of infection with T. gondii in blood was 51.5% when used latex agglutination test, also agree with results of [18] who confirm the percentage of infection was 49.7% when used LAT method as screening test for detection of Toxoplasmosis. LAT provides an excellent format for routine serological screening because of its high specificity, low cost and easy to use, but this method distinguish immunoglobulin cannot classes and is not helpful in cases of suspected congenital infection when need to distinguish between maternal IgG from infants IgG response[19].

Enzyme linked immunosorbent assay also used for detection of IgM and IgG antibodies in students sera, figure (1) showed the serum antibody of *Toxoplasma gondii* were detected in 24/112 of healthy student, IgG was positive in 15/24 (13.9) sample (37.5% in male and 25% in female) whereas IgM positive in 9 of 24(8.0%) sample (29.1%) in male and 8.3% in female). Our finding is in agree with results of [6] and [10] who confirm that 25.83% was seropositive for IgG and 4.16% for IgM when using **ELISA** test for detection on Toxoplasmosis in blood of pregnant and aborted women. also study of [11] showed that (11.9%) of healthy apparently students was seropositive to IgG antibody. IgM antibody test is still used by most laboratories to determine if a patient has been infected recently or in the distant past. In patient with recently acquired primary initially T.gondii specific IgM antibodies are detected initially and these titers become negative within a few months[20]. The finding could be explained by the fact the presence of IgG positive students were infected with latent toxoplasmosis.



Figure 1: levels of anti-toxoplasmosis IgG and IgM antibodies in serum of students

Molecular method also used to detect *T.* gondii DNA in blood, the results of our experience by using nested PCR showed that *T.* gondii DNA was found in 16.9% of healthy students (Figures 2 and 3). the our results were similar to results of [6] who use nPCR method for diagnosis of Toxoplasmosis among couples in Al-Ramadi. Many studies have demonstrated that PCR methods could actually detected Toxoplasmosis in blood specimens, PCR has allowed detection of *T.* gondii DNA in brain, tissue, CSF, urine, aminotic fluid and peripheral blood. Among PCR assay, the B1gene is consistent determined to be a useful target B1 gene is a tanden-arrayed 35-fold-repititive gene which has been used for both detection and typing of Toxoplasma strain in clinical samples. All Elisa and LAT results were higher than those of nPCR, this indicate that the presence of toxoplasma antibodies was an insufficient method to detection of toxoplasmosis in healthy students , it can be concluded that serological methods combined with the PCR method is recommended tool accurate diagnosis of *T.gondii.* 

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**Figure 2:** Agarose gel electrophoresis of n-PCR product using outer primer for detected B1gene in *T. gondii*, electrophoresis was performed on 1.5% agarose gel and run with a 5volt/cm for 1:30 hr. stained with Ethidium Bromide . M:ladder 100 bp, lane (7) negative control, lanes (2 and 6) negative samples, lanes (1,3,4,5,8,9,10,11,12,13,14 and 15) positive samples



**Figure 3:** Agarose gel electrophoresis of n-PCR product using Inner primer for detected B1gene in *T. gondii*, electrophoresis was performed on 1.5% agarose gel and run with a 5volt/cm for 1:30 hr. stained with Ethidium Bromide. M: ladder 100 bp, lane (7) negative control, lanes (2 and 6) negative samples, lanes (1,3,4,5,8,9,10,11,12,13,14 and 15) positive samples

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