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# DIAGNOSTIC STUDY OF TOXOPLASMA GONDII IN BLOOD DONOR AND APPLICANT FOR MARRIAGE OF THI-QAR UNIVERSITY-IRAQ BY REAL-TIME PCR

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**Abstract:** A rare intracellular parasite called Toxoplasma gondii infects a sizable fraction of the global population, although it seldom results in serious illness. Venous blood samples were obtained from 300 individuals (160 blood donors and 140 marriage applicants) who appeared to be in good health. The individuals ranged in age from 17 to 42 years old and had been treated for toxoplasma gondii infection in Thi-Qar for both sexes between October 2023 and the end of January 2024 using an enzyme-linked immunosorbent assay (ELISA). was employed to detect the B1 gene of T.gondii DNA using Real-Time Polymerase Chain Reaction (RT-PCR) and assess the existence of anti-Toxoplasma IgM and IgG antibodies .The findings demonstrated that, when using the ELISA antibody IgG, the overall toxoplasma gondii positive rate was 33%, and when utilizing the antibody for blood donors, it was 3%. For blood donors, the positive rate for antibodies was 28%, and for marriage applicants, the positive rate for antibodies was 5%.Twenty positive cases of anti-Toxoplasma antibodies were found in any positive blood sample with IgG and IgM, respectively. The ability to detect recent or active toxoplasma in applicants through marriage and blood donation in real time is advantageous.

**Keywords:** B1 gene, Toxoplasma, Real-time PCR.

## Introduction

Toxoplasma gondii is an obligatory intracellular tissue protozoan parasite that can infect humans and other warm-blooded domestic and wild animals, causing toxoplasmosis. The parasite is thought to infect almost one-third of the global population, and its distribution is widespread (1). Because they are frequently hunted by felids, birds can be thought of as an important reservoir of T. gondii. Because they are voracious eaters, they multiply uncontrollably and may contaminate food waste with T. gondii. Furthermore, they may serve as suitable hosts for this coccidian because they can fly great distances and feed on the ground (2. Using a PCR-based molecular technique, the direct identification of parasite-specific DNA in biological materials has become a prominent way for diagnosing T. gondii infection. Comparing molecular diagnostics to traditional procedures, it is both more economical and sensitive (3).PCR is used in molecular methods to specifically detect and analyze T. gondii DNA. These techniques have been used to a range of clinicand human (4)(5) T. gondii B1 gene DNA has been amplified and quantified using real-time PCR (6), (7) Real-Time PCR has been used to amplify and quantify T. gondii B1 gene DNA (8). During the PCR extension phase, Real-Time PCR cleaves a non-extendible, fluorescence-labeled hybridization probe using the 59 nuclease activity of Tag DNA polymerase (9). The fluorescent dye is typically tetramethyl-rhodamine-6-carboxy. In this paper, we outline the creation of a real-time quantitative PCR for T. gondii detection. This

methodology will make it easier for clinical laboratories to diagnose T. gondii infections. al samples from animals and have shown to be straightforward, sensitive, repeatable, and economical

**Material and methods:**

Sample collection involves taking blood samples from each individual blood donor and marrying the applicant from a bank and marriage facility in Thi-Qar.The drawn blood samples from each subject were transferred to the laboratory in sterile, clean test tubes with anticoagulant added for extracting DNA and without anticoagulant for collecting serum. Samples of clotted blood were refrigerated for the entire night. Serum was separated by centrifugation for 15 minutes at 3000 rpm, and it was then kept at -20 °C until analysis.In order to extract genomic DNA, additional non-clotted blood samples were kept in a refrigerator at 20 degrees Celsius.

**Serological test**:

The serum of ll cases were tested for the presence of specific IgM and IgG anti –Toxoplasma antibodies via ELISA kits (HMG Diagnostic Company , Germany ) according to the manufacture sinstructions.

**Isolation of genomic DNA from whole blood:**

Acommercial purification technique (Reagent Genomic DNA extraction kit; Invitrogen, USA) was used to extract DNA from whole blood samples of blood donors and applicants, adhering to the manufacturer's instructions for blood DNA purification .Purified DNA molecules were kept at -80C after the purity of the DNA concentration was estimated. The extracted genomic DNA from whole blood samples was examined using a Nano Drop spectrophotometer (THERMO, USA), which measures the absorbance at 260/280 to determine the DNA purity.

**Estimation of extracted total DNA**

Using a Nanodrop (Thermo Scientific Nano-drop Lite UV Visible Spectrophotometer, USA) to measure DNA content (ng/µL) and assess RNA purity at absorbance (260/280 nm), the extracted total DNA was examined as follows:

1. After launching the Nanodrop application, select the relevant application (DNA, nucleic acid).
2. The measurement pedestals were repeatedly cleaned with a dry wipe. Subsequently, 2µl of free nuclease water was carefully pipetted and deposited onto the lower measurement pedestals' surface to blank the apparatus.
3. A 1µl DNA sample was measured after the Nanodrop sampling arm was lowered

**Primer**

Real-Time PCR based TaqMan probe was performed for rapid detection of *T. gondii* according to method described by Meihuilin *et al*. (Fernanda *et al*.,2010). Real-Time PCR TaqMan probe and primers were used for amplification of conserved region B1 gene in *T. gondii*. These primers were provided by (Bioneer Company. Korea) as showed in table (1).

Table 1: Real-Time PCR TaqMan probe and primer

| Primer | Sequence | | Product size |
|---|---|---|---|
| B1 primer | F | TCCCCTCGCTGGCGAAAAGT | 94bp |
| | R | AGCGTTCGTGGTCAACTATCGATTG | |
| B1 probe | 5-6FAM-TCTGTGCAACTTTGGTGTATTCGCAG-TAMRA-3 | | |

### Real-Time PCR master mix preparation

AccuPower® DualStarTM qPCR PreMixBioneer, Korea) was used to perform the Real-Time PCR amplification reaction, and each sample's qPCR master mix was made in accordance with business instructions as shown in table (2):Table 2: The qPCR master mix

| RT-PCR master mix | Volume |
|---|---|
| DNA Template | 5 µL |
| Forward B1 gene primer (20pmol) | 2.5 µL |
| Reverse B1 gene primer (20pmol) | 2.5 µL |
| TaqMan B1 gene probe (20 pmol) | 2.5 µL |
| DEPC water | 37.5 µL |
| Total | 50 µL |

### Real-Time PCR Thermocycler Conditions

The dNTPs, Taq DNA polymerases, and 10X buffer for TaqMan probe amplification were already present in the Accu Power® Dual Star TM q PCR Pre Mixtubes when these qPCR master mix reaction components were introduced. After that, the tubes were placed in an Exispin vortex centrifuge and spun at 3000 rpm for three minutes. They were then moved into a Mini Opticon Real-Time PCR system, where they were subjected to the thermocycler settings listed in the following table (3):

**Table 3: Real-Time PCR Thermocycler condition**

| Steps | Condition | Cycle |
|---|---|---|
| Pre-Denaturation | 95 °C 5 min | 1 |
| Denaturation | 95 °C 20 sec | 45 |
| Annealing/ Extension | 60 °C 30 sec | |
| Detection (Scan) | | |

### Statistical analysis:

Data were analyzed with chi –square and p value 0.05 was considered statistically

### Results:

In order to determine the presence of Toxoplasma gondii infection using ELISA and Real-Time Polymerase Chain Reaction, the current study examined 300 blood donors who appeared to be in good health and 160 marriages for applicants from the center bank. The ELISA test results showed that, overall, the prevalence of Toxoplasma gondii was 70 (21.94) among those who were 24 (7.52%) blood donors and 46 (14.42%) applicants who were married. Using Real-Time quantitative PCR, the total prevalence of Toxoplasma gondii was found to be 20 (28.57), of which 8 (11.42%) were blood donors and 12 (17.14%) were applicants for marriage. The results are displayed in the table.

**Table (4) The central bank's percentage distribution of blood donors and marriage applicants with toxoplasmosis infection varies depending on the type of test.**

| Test type | Blood donor | | | Marriage for applicant | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | NO | +ve | | NO | +ve | | NO | +ve | |
| | | NO | % | | NO | +ve | | NO | % |
| ELISA test | 140 | 38 | 12.6% | 160 | 32 | 10.6 % | 300 | 70 | 23.33 |
| Real –time PCR | 26 | 8 | 11.42% | 46 | 12 | 17.14 | 70 | 20 | 28.57 |

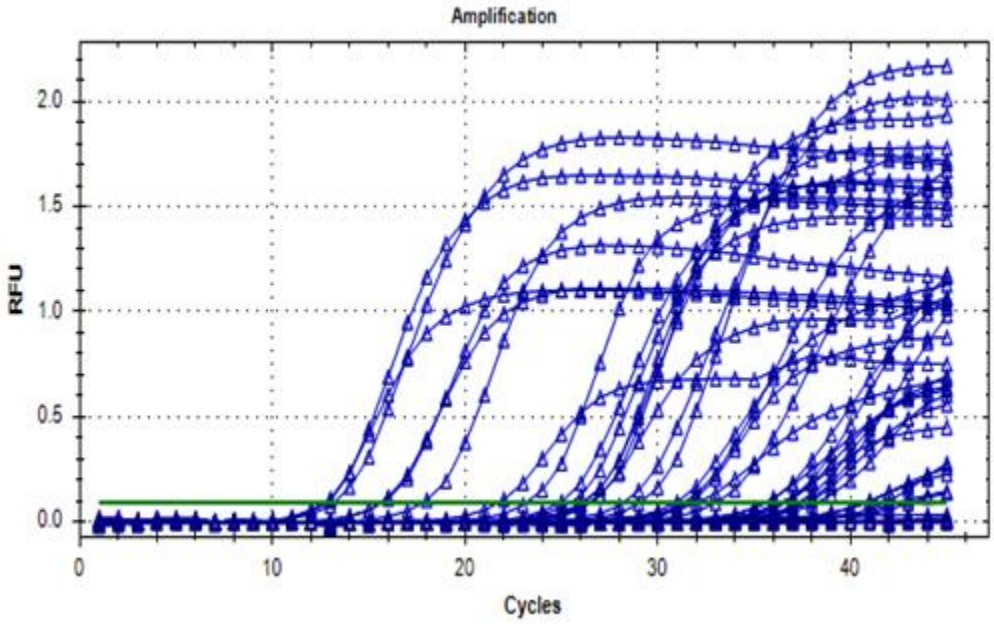


Figure 1: Real-time amplification plot of the B1 gene in T. gondi from a blood donor's sample and the applicants' marital status. Where Toxoplasma gondii DNA is 20 positive cases on the blue plot

**Comparison between Detection of *T. gondii* by ELISA (IgG and IgM) and Real-Time PCR**

**1. percentage of T. gondii infections based on residency as determined by blood donor IgG and IgM in ELISA and Real-Time PCR.**

Table 5 of the current investigation demonstrated Results of an ELISA and real-time PCR were provided for a study including both urban and rural housing types. In each group, the results revealed different percentages of negative, IgM positive, and IgG positive patients. There were 4.1% IgM positive, 20.4% IgG positive, and 75.5% negative cases among urban dwellers. There were 18.0% IgG positive, 2.3% IgM positive, and 71.42% negative cases among rural inhabitants. In all, 72.85% of the findings were negative, 2.8% were IgM positive, and 18.6% were IgG positive across all households. The given text contains values specific to each test, such as degrees of freedom and values, as well as statistical data for two tests: the Tabx2 and the Calx2.

**Table (5): percentage of T. gondii infection dependent on residency determined by blood donor IgG and IgM in ELISA and Real-Time PCR**

| ELISA | | | | | Real –Time PCR | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Residence | IgG Positive N&% | IgM Positive N&% | Negative N&% | Total N&% | Residence | IgG Positive N&% | IgM Positive N&% | Negative N&% | Total N&% |
| Urban | 10 20.4% | 2 4.1% | 37 75.5% | 49 100.0% | Urban | 2 8.3% | 0 0.0% | 22 91.7% | 24 100.0% |
| Rural | 23 18.0% | 3 2.3% | 65 71.42% | 91 100.0% | Rural | 5 10.9% | 1 2.2% | 40 87.0% | 46 100.0% |
| Total | 33 18.6% | 5 2.8% | 102 72.85% | 140 100.0% | Total | 7 10.0% | 1 1.4% | 62 88.6% | 70 100.0% |

Calx2:0.57 Tabx2;9.21 df:2 p- value:0.75 Calx2:0.66 Tabx2 :9.21 df: 2 p-value o.71

**2.. percentage of T. gondii infections based on residency determined by ELISA and real-time PCR utilizing IgG and IgM in marriage applicants**

Table (6), the present study showed Real-Time PCR and ELISA tests were conducted to determine the presence of IgM and IgG antibodies in individuals residing in urban and rural areas. The results showed that out of 24 individuals tested, 21 were negative, and 3 tested positive for IgG. In urban areas, out of 61 individuals tested, 51 were negative, and 9 tested positive for IgG. In rural areas, out of 46 individuals tested, 37 were negative, and 8 tested positive for IgG. The overall results from both urban and rural areas showed that out of 160 individuals tested, 128 were negative, and 29 tested positive for IgG The text gives the results of Chi-Carré tests with various values for Calx2, Tabx2, degrees of freedom (df), and p values. The results display numerical values unique to each test run.

**Table (6): Percentage of infected *T. gondii* based on residency trough using IgG and IgM in ELISA and Real-Time PCR in Applicants for marriage**

| ELISA | | | | | Real –Time PCR | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Residence | IgG Positive N&% | IgM Positive N&% | Negative N&% | Total N&% | Residence | IgG Positive N&% | IgM Positive N&% | Negative N&% | Total N&% |
| Urban | 9 14.8% | 1 1.6% | 51 83.6% | 61 100.0% | Urban | 3 12.5% | 0 0.0% | 21 87.5% | 24 100.0% |
| Rural | 20 20.2% | 2 2.0% | 77 77.8% | 99 100.0% | Rural | 8 17.4% | 1 2.2% | 37 80.4% | 46 100.0% |
| Total | 29 18.1% | 3 1.9% | 128 80.0% | 160 100.0% | Total | 11 15.7% | 1 1.4% | 58 82.9% | 70 100.0% |

Calx2:0.80 Tabx2:9.21 df:2 p- value:0.66 Calx2:0.85 Tabx2 :16. 81 df:6 p-valueo.65

**3. percentage of T. gondii infection based on gender as determined by blood donor ELISA and Real-Time PCR utilizing IgG and IgM.**

Table (7), the present study showed Real-Time PCR ELISA results showed that out of 46 total cases, 39 were negative, 1 was IgM positive, and 6 were IgG positive. Among males, out of 132 cases, 97 were negative, 5 were IgM positive, and 30 were IgG positive. For females, out of 24 cases, 23 were negative, and 1 was IgG positive. The overall results for gender distribution indicated that out of 70 males, 62 were negative, 1 was IgM positive, and 7 were IgG positive. The total number of cases analyzed was 140, with 102 negative, 5 IgM positive, and 33 IgG positive cases. The provided text includes statistical results for the Calx2 and Tabx2 tests, together with specific values for df and p-value. The results show different numbers for Calx2 (1.12 and 1.98) and constant values for Tabx2 (9.21), with variances in the p-values (0.56 and 0.37).

**Table (7): percentage of T. gondii infection based on gender as determined by blood donor ELISA and Real-Time PCR utilizing IgG and IgM.**

| ELISA | | | | | Real –Time PCR | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Gender | IgG Positive N&% | IgM Positive N&% | Negative N&% | Total % | Gender | IgG Positive N&% | IgM Positive N&% | Negative N&% | Total N&% |
| male | 30 22.7% | 5 3.8% | 97 73.5% | 132 100.0% | male | 6 13.0% | 1 2.2% | 39 84.8% | 46 100.0% |
| Female | 3 37.5% | 0 0.0% | 5 62.5% | 8 100.0% | female | 1 4.2% | 0 0.0% | 23 95.8% | 24 100.0% |
| | 33 | 5 | 102 | 140 | Total | 7 | 1 | 62 | 70 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Total | 23.6% | 3.6% | 72.9% | 100.0% | | 10.0% | 1.4% | 88.4% | 100.0% |

Calx2:1.12 Tabx2:9.21 df:2 p- value:0.56 Calx2:1.98 Tabx2 :9.21 df:2 p-value o.37

**4.. percentage of T. gondii infections based on gender as determined by Real-Time PCR and IgG and IgM in ELISA tests on marriage applicants**

The present investigation's Table 5 showed Data from real-time PCR and ELISA analyses were presented for a study that included both urban and rural housing types. The results showed varying percentages of negative, IgM positive, and IgG positive patients in each group. Among urban dwellers, there were 4.1% IgM positive cases, 20.4% IgG positive cases, and 75.5% negative cases. Among rural residents, there were 18.0% IgG positive cases, 2.3% IgM positive cases, and 71.42% negative cases. Across all families, 72.85% of the results were negative, 2.8% were IgM positive, and 18.6% were IgG positive. The provided text includes statistical information for the Tabx2 and Calx2 tests in addition to values unique to each test, such as degrees of freedom and values.

**Table (8) . ): The percentage of T. gondii infections dependent on gender as determined by Real-Time PCR and IgG and IgM in ELISA in marriage-seeking applicants.**

| ELISA | | | | | Real –Time PCR | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Gender | IgG Positive N&% | IgM Positive N&% | Negative N&% | Total % | Gender | IgG Positive N&% | IgM Positive N&% | Negative N&% | Total N&% |
| male | 10 14.3% | 1 1.4% | 59 84.3% | 70 100.0% | male | 2 8.3% | 0 0.0 | 22 91.7% | 24 100.0% |
| Female | 19 21.1% | 2 2.2% | 69 76.7% | 90 100.0% | female | 9 19.6% | 1 2.2% | 36 78.35 | 46 100.0% |
| Total | 29 18.1% | 3 1.9% | 128 80.0% | 160 100.0% | Total | 11 15.7% | 1 1.4% | 58 82.9% | 70 100.0% |

Calx2:1.43 Tabx2:9.21 df:2 p- value:0.48 Calx2:2.13 Tabx2 :9.21 df:2 p-value o.34

**5. percentage of T. gondii infection based on age group as determined by blood donor IgG and IgM in ELISA and Real-Time PCR**

Table (9), the present study Real-Time PCR and ELISA results were presented in the text. The data showed the breakdown of total samples tested, the percentage of negative results, and the presence of IgM and IgG antibodies in different age groups. The age groups ranged from 17-21, 22-26, 27-31, 32-36, 37-41, and more than 40. The results indicated varying percentages of negative, IgM positive, and IgG positive cases within each age group. Overall, the total number of samples tested was 140, with 102 negative results, 5 IgM positive results, and 33 IgG positive results. The provided text includes statistical results for two data groups, together with degrees of freedom (df) and Chi-square test values (Calx2). Additionally, p values are provided for each test that is run. The value of p in the first test is 0.71, whereas the value of p in the second test is 0.83. The findings show statistically significant differences between the groups of data that were analyzed.

**Table (9):. percentage of T. gondii infection by age group as determined by blood donor IgG and IgM in ELISA and Real-Time PCR**

| ELISA | | | | | Real-Time PCR | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group | IgG Positive N&% | IgM Positive N&% | Negative N&% | Total N&% | Group | IgG Positive N&% | IgM Positive N&% | Negative N&% | Total N&% |
| 17-21 | 2 18.2% | 0 0.0% | 9 81.8% | 11 100.0% | 17-21 | 0 0.0% | 0 0.0% | 8 100.0% | 8 100.0% |
| 22-26 | 3 16.7% | 2 11.1% | 13 72.2% | 18 100.0% | 22-26 | 1 7.7% | 0 0.0% | 12 92.3% | 13 100.0% |
| 27-31 | 5 22.7% | 1 4.5% | 16 72.7% | 22 100.0% | 27-31 | 2 16.7% | 0 0.0% | 10 83.3% | 12 100.0% |
| 32-36 | 7 35.0% | 1 5.0% | 12 60.0% | 20 100.0% | 32-36 | 2 14.3% | 0 0.0% | 12 85.7% | 14 100.05 |
| 37-41 | 7 23.3% | 1 3.35 | 22 73.3% | 30 100.0% | 37-41 | 1 5.3% | 1 5.35 | 17 89.5% | 19 100.0% |
| More than40 | 9 23.1% | 0 0.0 | 30 76.9% | 39 100.0% | 32-36 | 1 7.1% | 0 0.0% | 13 92.9% | 14 100.0% |
| Total | 33 23.6% | 5 3.6% | 102 72.9% | 140 100.0% | Total | 7 8.8% | 1 1.3% | 72 90.0% | 80 100.0% |

Calx2:7.11 Tabx2:23.21 df:10 p- value:0.71 Calx2:5.78 Tabx2 :23.21 df:10 p-value o.83

**6. Percentage of T. gondii infection by age group as determined by Real-Time PCR and IgG and IgM in ELISA testing on marriage applicants**

Table(10), the present study showed Real-Time PCR and ELISA tests were conducted on different age groups to determine the presence of IgM and IgG antibodies. The results showed varying percentages of negative and positive cases in each age group. The age groups ranged from 17-21 to more than 40 years old. The total number of individuals tested was 160, with a distribution of negative, IgM positive, and IgG positive cases across the different age groups. The highest percentage of positive cases was found in the age group of 37-41 years old, with 30% testing positive for IgG antibodies. Overall, the data presented a breakdown of test results by age group and antibody typeThe provided material includes statistical results about the values of p, degres lebrite (df), and Calx2 and Tabx2 values. The data provide specific numbers for Calx2 and Tabx2, together with related p values and degrees of freedom. The results show different numbers for Calx2 and Tabx2, with different values of p for each measure.

**Table (10):. percentage of T. infection based on age determined by ELISA and Real-Time PCR utilizing IgG and IgM in marriage-seeking applicants**

| ELISA | | | | | Real-Time PCR | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group | IgG Positive N&% | IgM Positive N&% | Negative N&% | Total N&% | Group | IgG Positive N&% | IgM Positive N&% | Negative N&% | Total N&% |
| 17-21 | 6 13.6% | 1 2.3% | 37 84.1% | 44 100.0% | 17-21 | 1 12.5% | 0 0.0% | 7 87.5% | 8 100.0% |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 22-26 | 5<br>16.7% | 1<br>3.3% | 24<br>80.0% | 30<br>100.0% | 22-26 | 2<br>15.4% | 0<br>0.0% | 11<br>84.6% | 13<br>100.0% |
| 27-31 | 5<br>13.9% | 1<br>2.8% | 30<br>83.3% | 36<br>100.0% | 27-31 | 2<br>18.2% | 1<br>9.1% | 8<br>72.7% | 11<br>100.0% |
| 32-36 | 4<br>16.0% | 0<br>0.0% | 21<br>84.0% | 25<br>100.0% | 32-36 | 3<br>21.4% | 0<br>0.0% | 11<br>78.6% | 14<br>100.0% |
| 37-41 | 6<br>30.0% | 0<br>0.0% | 14<br>70.0% | 20<br>100.0% | 37-41 | 2<br>20.0% | 0<br>0.0% | 8<br>80.0% | 10<br>100.0% |
| More than40 | 3<br>50.0% | 0<br>0.0% | 3<br>50.0% | 5<br>100.0% | 32-36 | 1<br>7.1% | 0<br>0.0% | 13<br>92.9% | 14<br>100.0% |
| Total | 29<br>18.0% | 3<br>1.9% | 29<br>18.0% | 160<br>10.0% | Total | 11<br>15.7% | 1<br>1.4% | 58<br>82.9% | 70<br>100.05 |

Calx2:8.51 Tabx2:23.21 df:10 p- value:0.57 Calx2:6.89 Tabx2 :23.21 df:10 p-valueo.73

**Discussion:**

Real-Time PCR was employed in addition to serological diagnosis of T. gondii to confirm infection by detecting the B1 gene of T. gondii DNA in the blood donor and marriage for applicants. DNA from Toxoplasma gondii was effectively isolated, and Real-Time PCR analysis was performed .A number of PCR-based methods have been created as substitute measurements for T.gondi infection diagnosis. These methods employ the B1 gene repetitive sequence, which is the most conserved gene sequence among the various strains of T. gondii (10) ribosomal DNA, the (SAG1) gene, and others. With(11), the B1 gene was first used to detect T. gondii. Due to its specificity and sensitivity, the B1 gene—despite having an unclear function—is primarily used in a range of epidemiological and diagnosis research (12)(13). In Real-Time PCR, the cycle threshold value (CT) for positive samples varied from Ct 28 to Ct 37. The amount of template present at the beginning of the amplification reaction is the primary factor in determining the CT of a reaction.If there's a lot of template at the beginning of the reaction very few amplification cycles could be needed to gather enough product to provide a fluorescent signal that is more prominent than the background.As a result, the reaction will have an early or low Ct. In contact, additional amplification cycles will be needed for the fluorescence to rise above background if there is a tiny amount of template present at the beginning of the reaction. As a result, the reaction will have a late or high Ct. The quantitative component of real-time PCR is based on this relationship (14).According to the test, only 28.57 out of 70 applicants were blood donors, and of those, 8 (11.42%) were married (17.14%). Statistics show that there is no discernible difference between them (po.o5).While the rate was lower in this examination than those recorded by(15), who recorded that the rate infection of toxoplasmosis was (69%) other examine in Iraq (16)(17) and(18)), the positive result was higher in this study recorded by(19) in Turkey, who demonstrated that rate was (1.3%) and with(20) in Taiwan, who recorded that no active parasitemia was detected by Real-time assay. indicated the rarity of miscarried women (38.0%, 17.7%, 16%). The reason for these differences could be that specimens were only obtained from male blood donors, and as a result, the percentage of male applicants for marriage was not significant according to ELISA. Alternatively, it could be that neither males nor females were likely exposed to toxoplasmosis depending on how they lived or how well they followed hygiene practices. Number of infected individuals by age group; moreover, individuals may have been exposed to soil or have had childhood contact with toxoplasma through cats has caused varying percentages of anti-Toxoplasma antibodies to build in humans, which can result in a chronic toxoplasmosis infection (21).Given the variations in the specificity and sensitivity of the diagnostic method employed, as well as the host's individual response to the parasite strain, the discrepancies between the previous and current results suggest that the parasite strain may have a significant impact on the host's immune system's stimulation against the parasite (22).Despite the variation in sample size and cleanliness, Study 11 (3.26%) 11 positive cases of anti-Toxoplasma antibodies were detected, whereas no positive cases with IgG, IgM, or both IgM

and IgG were found. Those who recorded positive results in Real-Time analysis were dispersed on the patterns of the anti-Toxoplasma antibodies.This outcome was consistent with that of(20), who reported that 1783 blood samples from healthy donors were examined for the presence of T. gondii antibodies and DNA. 5 (0.28%) and 166 (9.3%) individuals tested positive for anti-Toxoplasma IgM and IgG, respectively, using ELISA and Real-Time PCR.(23) found no evidence of active parasitemia of anti-Toxoplasma IgG by real-time PCR assay, and no active parasitemia of positive ELISA result was discovered by the Real-Time assay. Furthermore, these results demonstrated that several studies have previously demonstrated that a positive serology result, which indicates local production of antibodies, does not necessarily accompany a PCR result (24),(18).

Since specific anti-Toxoplasma IgG or IgM may not be formed until several weeks after parasitemia, serological detection is currently the only method used to diagnose toxoplasmosis. This means that certain antibodies may not be detectable during the active period of T. gondii infection. In order to detect Toxoplasma gondii, we amplified the T. gondii B1 gene in this study using highly specific molecular methods such as primers and Real-Time PCR-based Taq Man probe (25).

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