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**Original Research Article**

# Prevalence and Serological Diagnosis of Toxoplasmosis in Two Regions (Karkh and Rusafa) of Baghdad Province

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**Abstract:** *Toxoplasma gondii* exhibits a global distribution and is among the highest common infectious parasite in Iraq. The current research aimed to assess the seroprevalence of toxoplasmosis across various demographic groups in two regions of Baghdad, Karkh and Rusafa. Venous blood samples were taken from 450 male and female students, both pre-marital and married. Serum was obtained from each individual for the identification of specific anti-*T. gondii* IgG and IgM utilizing LAT, ELISA-IgG test, ELISA-IgM test, and miniVIDAS-IgG test. The overall percentage of seropositive anti-*T.gondii* antibodies by LAT 152 is 43.4%. The overall percentages of seropositive anti-T. gondii by ELISA-IgG and miniVIDAS-IgG tests were 80 (50.0%) each, with no significant variations observed between the findings of both tests. The seroprevalence of anti-*T. gondii* antibodies detected by the ELISA-IgM test was 2 (1.3%). Significant differences ($P<0.05$) were observed in the results across different regions based on age groups using LAT, ELISA-IgG, and miniVIDAS-IgG tests. Conversely, no significant differences ($P>0.05$) were noted with ELISA-IgM in the age group of 19-23 years, which exhibited a high seroprevalence of toxoplasmosis. Females exhibited a substantial increase in the percentage of seropositivity for toxoplasmosis compared to male students, as determined by LAT, ELISA-IgG, and miniVIDAS-IgG tests. The prevalence of *T. gondii* infection in pre-marital females was substantially higher at 52 (49.4%) compared to married females at 8 (7.6%), as determined by ELISA-IgG testing. The prevalence of *T. gondii* infection in pre-marital females was substantially higher at 49 (46.6%) compared to married females at 10 (9.5%), as shown by miniVIDASIgG testing. The largest percentages of toxoplasmosis were observed in aborted females, with ELISA-IgG showing 3 (37.5%) and miniVIDAS-IgG showing 4 (40%), in contrast to normal females, which exhibited 5 (62.5%) and 6 (60%), respectively.

**Keywords:** Toxoplasmosis; Serological test; Latex; ELIZA; miniVIDAS.

## INTRODUCTION

A small North African rodent, Ctenodactylus gundi, is the source of toxoplasmosis (Smith *et al.,* 2021). The protozoan Apicomplexa causes the disease, which is common in humans and other animal species and has been documented in numerous countries with varying climates (Jiménez-Martín *et al.,* 2020). Felidae serve as both intermediate and definitive hosts, while all mammals, including humans and birds, are intermediate hosts. Meat from sheep and goats is a significant source of toxoplasmosis infection (Esubalew *et al.,* 2020). Humans can contract *T. gondii* by eating raw infected meat from intermediate hosts, such as sheep, goats, and cattle, or by consuming contaminated food or water that contains sporulated oocysts. Additionally, the virus is spread through the placenta (Gisbert Algaba *et al.,* 2020). Although the disease is usually benign, it can cause serious morbidity and mortality in immunocompromised people, particularly those who have acquired immunodeficiency syndrome, or AIDS (Almeria and Dubey, 2020), as well as in developing fetuses (Mata *et al.,* 2021). During the acute stage of infection, millions of oocysts are formed in the intestinal mucosa of cats, the last host, and may be expelled in the feces in a single day. Cats have been regarded as a health concern for both humans and wildlife

because they are recognized to be a source of the disease (Odeniran *et al.*, 2020). Breeders of sheep and goats suffer large financial losses as a result of the parasite, which is a leading cause of abortion (Dubey *et al.*, 2020). One of the major causes of abortion in humans is toxoplasmosis (Kalantari *et al.*, 2021).

Although parasite infection is common worldwide, seroprevalence varies significantly by nation and population group (Ducrocq *et al.*, 2021). In the absence of immunosuppression. T. gondii antibodies in mammal serum have been found using a variety of serologic techniques such as ELISA, or enzyme-linked immunosorbent assay, and miniVIDAS, or Vitek Immunodiagnostic Assay System. The most often used diagnostic tests are the latex agglutination test (LAT), modified agglutination test (MAT), indirect fluorescent antibody test (IFAT), Sabin-Feldman (SF) dye test, complement fixation test (CFT), and indirect haemagglutination test (IHAT) and the SF test which is the primary method for detecting toxoplasmosis in both humans and animals. Toxoplasma gondii infection is recognized by IgG, IgM, IgA and IgE antibodies in patients with acute and chronic toxoplasmosis depending on the strain and the stage of the parasite, therefore essential to estimate the time of infection as precisely as possible to properly manage the risk to the fetus of a maternal infection, A positive IgM antibodies result in a single serum specimen may reflect an acute infection.

**Aims of the study**

To determine the seroprevalence of anti-Toxoplasma antibodies (IgM and IgG) among male and female in different ages from two regions in Baghdad province. and then examine these samples by Latex then by miniVIDAS or ELISA is a more accurate test for calculating the percentage of toxoplasmosis infection and examine the connection between Toxoplasma infection and the many clinical parameters examined in this investigation.

**Historical Background**

Toxoplasmosis is a significant disease caused by the microscopic parasite *Toxoplasma gondii*. This disease was first identified in 1908 by Nicolle and Manceaux in North Africa, as well as by Splendore in Brazil. The species name derives from the North African rodent Ctenodactylus gondii, from which the parasite was initially isolated (Ajioka and Morrissette, 2009). The genus name is rooted in the Greek word toxon, meaning "bow," which refers to the organism's crescent shape (Michael and John, 2000). Toxoplasmosis was first reported in humans by Junku in 1923, who discovered parasitic cysts in the retina of a child suffering from congenital hydrocephalus (Remington and Desmonts, 1990).

In Iraq, the parasite was first identified by Machattie (1938) through smears taken from the spleens and lungs of two street dogs in Baghdad.

**Stages of *Toxoplasma gondii***

There are three stages of *T. gondii*:-

1. Oocysts, which give rise to sporozoites.
2. Tachyzoite or trophozoite, the actively proliferating form.
3. Cystozoite or bradyzoite, the resting form.

**The Life Cycle of Toxoplasmosis:**

The life cycle of *Toxoplasma gondii* consists of stages occurring in both feline and non-feline hosts, which are associated with sexual and asexual development, respectively.

**Transmission of T. gondii:**

There are multiple pathways for parasite transmission:

1- Transplacental transmission (Maternal transmission).
2- Oral Transmission.
3- Blood Transfusion.

**Diagnosis of Toxoplasmosis:**

The diagnostic laboratory procedures for toxoplasmosis include the isolation of the organism and the detection of antibodies to Toxoplasma in the patient's serum. Furthermore, a cutaneous response akin to the tuberculin reaction may be elicited in positive patients using an antigen derived from Toxoplasma (Sharma *et al*. 2023). The clinical pathology of the disorder provides minimal diagnostic assistance; nevertheless, during the acute phase, an elevation in cells and protein in the cerebrospinal fluid may be observed, although an increase in protein without a corresponding rise in cells is occasionally noted in seemingly resolved instances. No specific blood changes or eosinophilia have been documented.

**1- Direct Microscopic Examination:**

Tissues obtained by biopsy or autopsy can undergo direct microscopic inspection by being fixed, sectioned, and stained, or by creating impression smears treated with polychrome stains. Suspected fluid may undergo centrifugation, and the sediment can be analyzed in stained smears. Cerebrospinal and ventricular fluid must be analyzed promptly following

aspiration. Toxoplasma gondii can be extracted from the placenta, umbilical cord, or newborn blood through inoculation into mice or cell culture.

**2- Serological Diagnosis:**

The predominant technique employed to identify toxoplasmosis is serologic testing for Toxoplasma-specific immunoglobulin G (IgG) antibodies. Toxoplasma immunoglobulin M (IgM) antibody titers increase shortly after infection but generally decrease rapidly and become undetectable within weeks or months. IgG titers increase within one to two months post-infection and persist elevated for a lifetime. The presence of toxoplasma IgG antibodies signifies an existing T gondii infection, whereas IgM antibodies indicate a recent infection. Serum antiToxoplasma antibody titers can be assessed using many approaches, including the following:

**A- Dye Test (D.T.):** It is referred to as Sabin-Feldman. The dye test was developed in 1948 by Sabin and Feldman (Jeske *et al.*, 2024). The DT is the definitive serological assay for Toxoplasma antibodies in humans. Live Toxoplasma tachyzoites are treated with a complement-like accessory factor and test serum at 37°C for one hour prior to the addition of methylene blue. A specific antibody enhances membrane permeability in the parasite, allowing cytoplasmic leakage, resulting in the tachyzoite's inability to absorb the dye, therefore appearing colorless. Tachyzoites that are not exposed to a specific antibody (i.e., a negative serum sample) absorb the dye and exhibit a blue coloration. The DT is both specific and sensitive in humans, but may be unreliable in other species.

**B- Indirect Hemagglutination Assay (IHAT**): Jacobs and Lunde described an indirect hemagglutination test (IHAT) utilizing sheep red cells as an antigen carrier (Ehrens *et al.,* 2020). The reagent consists of a suspension of stabilized erythrocytes coated with a pure antigen derived from Toxoplasma gondii, incubated in mouse peritoneal exudate. These erythrocytes interact with certain antibodies found in human or animal serum, thereby creating a uniform network on the plate (positive reaction). In the absence of specific antibodies, erythrocytes aggregate to form a distinct button at the bottom of the plate, indicating a negative reaction. This method is applicable to both humans and animals, proving efficient for testing large quantities of sera; however, it is not suitable for detecting congenital and neonatal infections, and it is subject to variability in red blood cell quality and antigen differences.

**C- Complement Fixation Test (CFT):** The complement fixation test (CFT) is the fundamental technique for diagnosing toxoplasmosis (Nova *et al.,* 2023). Antigens were extracted from the peritoneal exudates of infected mice and guinea pigs. False positives may occasionally occur in malaria, TB, salmonellosis, and trypanosomiasis. This test is infrequently utilized currently due to technological challenges and insufficient sensitivity.

**D- Latex Agglutination Test (LAT):** The toxoplasmosis latex reagent is a dispersion of polystyrene particles sensitized with Toxoplasma gondii antigens (Angarita-Corzo *et al.*,2025). When serum or plasma from an infected individual is combined with latex particles, a characteristic agglutination pattern is seen due to the development of antigen-antibody complexes. In the absence of infection, no agglutination will occur. A positive result shows the presence of antibodies.

**E- Indirect Fluorescent Antibody Test (IFAT):** The IFAT is a straightforward and often utilized technique. Whole, killed Toxoplasma tachyzoites are incubated with diluted test serum, followed by the addition of the appropriate fluorescent antispecies serum, and the outcome is subsequently examined using a fluorescence microscope (Voyiatzaki *et al.*,2024). Commercially accessible fluorescent-labelled antibodies exist for various animal species; the procedure is very cost-effective, and kits can be purchased. Nonetheless, the technique necessitates a fluorescent microscope, and the results are interpreted visually, which may lead to individual variability. Locating certain species-specific conjugates may prove challenging, and there exists a potential risk of cross-reactivity with rheumatoid factor.

**F- Enzyme-Linked Immunosorbent Assay (ELISA):** Ban Waeman and Schurs conducted research in Holland in 1971, whereas Engavell Perlamann originally created this test in Sweden in 1972. The test is highly sensitive and utilizes an enzyme coupled with either the antibody or the antigen to measure the respective antigens and antibodies (Voyiatzaki *et al.*,2024). This is a straightforward, quick, and precise method for detecting IgM antibodies in persons with acute acquired toxoplasmosis and for assessing whether pregnant women were infected during gestation or before to conception. IgM generally appears within the first week of infection, rises rapidly, and thereafter decreases at varied rates, eventually disappearing after a few months. IgM antibodies may persist for years beyond the acute infection, and the reliability of commercially available assays varies significantly. The IgM-ELISA identifies recently acquired acute Toxoplasmosis and congenital infection. Positive ELISA-IgG reactivity signifies a prior infection, regardless of its regency or duration. Positive IgA reactivity serves as a reliable indicator of recent active infection and can confirm primary, congenital (maternal, fetal, and neonatal), and reactivating infections. Immunosuppressed individuals may exhibit diminished IgA responses.

**G- VITEK Immunodiagnostic Assay System (miniVIDAS):** The miniVIDAS is referred to as a "multiparametric" instrument. The phrase "multiparametric" describes the capabilities of the VIDAS. The user can execute compatible tests using identical protocols inside the same area. Salmonella, Listeria, and E. coli 0157 can be processed concurrently in the same section. The test principle integrates a two-step enzyme immunoassay sandwich technique with a concluding fluorescence detection method (ELFA), specifically utilizing mini VIDAS. It is a straightforward, quick, and precise method for quantifying anti-toxoplasma IgM or IgG in individuals with acute or chronic

toxoplasmosis. The VIDAS apparatus executes all procedures autonomously. This technique exhibits great specificity for the identification of IgM and IgG (Fiedler *et al.*, 1999).

## MATERIALS AND METHODS

**Subject selection and blood sample collection:**

Blood samples were collected from 350 peoples (males and females) from two regions in Baghdad province (Karkh and Rusafa) with age ranging from 19 to 28 years. Five ml of venous blood were collected from each person by using disposable syringe. The blood sample was placed in a plain tube and left stand for 20 minutes at room temperature to clot. Serum was separated from clot by centrifugation at 3000 rpm for 10 min of (5ml), Then the obtained serum was divided into three portions in different appendrof tubes to avoied repeated freezing and thawing, and stored at -20ºC until being analyzed for detection of Toxoplasma antibodies by different serological tests such as:

Latex Agglutination Test (LAT), Enzyme Linked Immunosorbant Assay (ELISA- IgG and ELISA–IgM) and VITEK Immuno –diagnostic Assay System (miniVIDAS)

## RESULTS AND DISCUSSION

**Table 1: The percentage distribution of LAT sero +ve and sero –ve of peoples from two different regions in Baghdad province**

| TEST / Result | | LAT | | |
|---|---|---|---|---|
| | | LAT + ve | LAT – ve | Total |
| Karkh | NO | 59 | 97 | 156 |
| | % | 16.8 | 27.7 | 44.5 |
| Rusafa | NO | 93 | 101 | 194 |
| | % | 26.6 | 28.9 | 55.5 |
| Total | No | 152 | 198 | 350 |
| | % | 43.4 | 56.6 | 100 |
| P-value | | 0.0378 * | 0.894 NS | 0.0377 * |
| * (P≤0.05). | | | | |

The over all percentage of seropositive anti-*T. gondii* antibodies by LAT 152 (43.4%). There is significant differences (P≤0.05) showed in results between negative and positive samples in the two different regions.

**Table 2: The percentage distribution of toxoplasmic peoples according to the age groups from two regions in Baghdad province by latex agglutination test (LAT)**

| Test / Age Groups(year) | Latex test | | | | | |
|---|---|---|---|---|---|---|
| | Latex+ve | | Latex-ve | | Total | |
| | NO | % | NO | % | NO | % |
| 19-23 | 82 | 23.4 | 109 | 31.1 | 191 | 54.5 |
| 23-25 | 47 | 13.4 | 53 | 15.2 | 100 | 28.6 |
| 25-28 | 23 | 6.6 | 36 | 10.3 | 59 | 16.9 |
| Total | 152 | 43.4 | 198 | 56.6 | 350 | 100 |
| P-value | -- | 0.0001 ** | -- | 0.0001 ** | -- | 0.0001 ** |
| ** (P≤0.01). | | | | | | |

There was a high significant differences (P≤0.01) showed in results of different peoples according to the age groups by LAT.

**Table 3: The distribution of LAT sero +ve in each studied region according to the age groups**

| Test / Age Groups | LAT +ve | | | | | |
|---|---|---|---|---|---|---|
| | Karkh | | Rusafa | | Total | |
| | NO | % | NO | % | NO | % |
| 19-23 | 27 | 7.7 | 55 | 15.7 | 82 | 23.4 |
| 23-25 | 19 | 5.5 | 27 | 7.7 | 46 | 13.2 |
| 25-28 | 12 | 3.4 | 12 | 3.4 | 24 | 6.8 |
| Total | 58 | 16.6 | 94 | 26.8 | 152 | 43.4 |
| P-value | -- | 0.267 NS | -- | 0.0083 ** | -- | 0.0051 ** |
| ** (P≤0.01). | | | | | | |

The over all percentage of seropositive anti-*T. gondii* antibodies by LAT in Karkh 58 (16.6%) and in Rusafa 94 (26.8%) and there was high significant differences (P≤0.01) between two regions according to the ages group.

**Table 4: The distribution of LAT sero +ve in each studied regions according to the sex**

| Test / Sex | LAT sero +ve | | | | | |
|---|---|---|---|---|---|---|
| | Karkh | | Rusafa | | Total | |
| | NO | % | NO | % | NO | % |
| Male | 16 | 4.6 | 31 | 8.8 | 47 | 13.4 |
| Female | 43 | 12.3 | 62 | 17.7 | 105 | 30 |
| Total | 59 | 16.9 | 93 | 26.5 | 152 | 43.4 |
| P-value | -- | 0.0001 ** | -- | 0.0001 ** | -- | 0.0001 ** |
| ** (P≤0.01). | | | | | | |

Female samples scored by a high significant (P≤0.01) increase in percentages of sero+ve of toxoplasmosis than males students by LAT.

**Table 5: The distribution of LAT sero +ve in each studied regions according to the females group**

| **Test / Female groups** | **LAT sero +ve** | | | | | |
|---|---|---|---|---|---|---|
| | **Karkh** | | **Rusafa** | | **Total** | |
| | **NO** | **%** | **NO** | **%** | **NO** | **%** |
| Premarital | 30 | 8.7 | 45 | 12.8 | 75 | 21.5 |
| Married | 10 | 2.8 | 20 | 5.7 | 30 | 8.5 |
| Total | 40 | 11.5 | 65 | 18.5 | 105 | 30 |
| P-value | -- | 0.0016** | -- | 0.0019** | -- | 0.0001 |
| ** (P≤0.01). | | | | | | |

The highest percentage of toxoplasmosis were found in premarital female and the was highly significant differences (P≤0.01) according to the females groups.

**Table 6: The percentages of toxoplasmosis in peoples in two regions in Baghdad province by to ELISA –IgG-IgM antibody test**

| Test / Test | ELISA- (Ab) | | | | | |
|---|---|---|---|---|---|---|
| | Karkh | | Rusafa | | Total | |
| | NO | % | NO | % | NO | % |
| ELIZA IgG + | 27 | 17.7 | 50 | 32.8 | 77 | 50.6 |
| ELIZA IgM + | 2 | 1.3 | 1 | 0.7 | 3 | 2 |
| Total | 29 | 19 | 51 | 33.6 | 80 | 52.6 |
| P-value | -- | 0.0001 ** | -- | 0.0001 ** | -- | 0.0001 ** |
| ** (P≤0.01). | | | | | | |

There was high significant differences (P≤0.01) between two regions according to the ELISA IgM and IgG test.

**Table 7: The seroprevalence of toxoplasmosis ELISA-IgG (Ab) positive samples according to the age group between the two regions in Baghdad province**

| Test / Age Groups | ELISA–IgG (Ab) | | | | | |
|---|---|---|---|---|---|---|
| | Karkh | | Rusafa | | Total | |
| | NO | % | NO | % | NO | % |
| 19-23 | 15 | 9.8 | 24 | 15.8 | 39 | 25.6 |
| 23-25 | 10 | 6.6 | 15 | 9.8 | 25 | 16.4 |
| 25-28 | 5 | 3.3 | 11 | 7.3 | 16 | 10.6 |
| Total | 30 | 19.7 | 50 | 32.9 | 80 | 52.6 |
| P-value | -- | 0.084 NS | -- | 0.0086 ** | -- | 0.0065 ** |
| ** (P≤0.01). | | | | | | |

There was high significant differences (P≤0.01) between two regions according to the ages group by ELISA IgG test.

**Table 8: The seroprevalence of toxoplasmosis by miniVIDAS-IgG (Ab) positive samples according to the age group between the two regions in Baghdad province**

| Test / Age Groups | miniVIDAS–IgG (Ab) | | | | | |
|---|---|---|---|---|---|---|
| | Karkh | | Rusafa | | Total | |
| | NO | % | NO | % | NO | % |
| 19-23 | 17 | 11.2 | 24 | 15.7 | 41 | 26.9 |
| 23-25 | 10 | 6.5 | 16 | 10.6 | 26 | 17.1 |
| 25-28 | 4 | 2.6 | 9 | 5.9 | 13 | 8.5 |
| Total | 31 | 20.3 | 49 | 32.2 | 80 | 52.2 |
| P-value | -- | 0.0382* | -- | 0.0084** | -- | 0.0051** |
| *(P≤0.05), **(P≤0.01) | | | | | | |

There was significant differences (P≤0.05) in karkh region according to the ages groups by miniVIDAS-IgG test and highly significant differences (P≤0.01) in Rusafa region according to the age by the same serological test.

**Table 9: The percentage of toxoplasmosis in different peoples according to the age groups by ELISA-IgG and miniVIDAS-IgG (Ab) test**

| Age Groups (years) | ELISA-IgG and miniVIDAS- IgG (Ab) | | | | | |
|---|---|---|---|---|---|---|
| | ELISA + | | miniVIDAS + | | Total | |
| | NO | % | NO | % | NO | % |
| 19-23 | 39 | 24.4 | 41 | 25.6 | 80 | 50.0 |
| 23-25 | 25 | 15.6 | 26 | 16.3 | 51 | 31.9 |
| 25-28 | 16 | 10 | 13 | 8.1 | 29 | 18.1 |
| Total | 80 | 50.0 | 80 | 50.0 | 160 | 100.0 |
| P-value | -- | 0.0001 ** | -- | 0.0001 ** | -- | 0.0001 ** |
| ** (P≤0.01). | | | | | | |

There was highly significant differences (P≤0.01) according to the age by both ELISA IgG and miniVIDAD IgG test.

**Table 10: The percentage of toxoplasmosis in different peoples according to the sex by ELISA-IgG and miniVIDAS-IgG (Ab) test**

| Test / Sex | ELISA-IgG and miniVIDAS- IgG (Ab) | | | |
|---|---|---|---|---|
| | ELISA + | | miniVIDAS + | |
| | NO | % | NO | % |
| Male | 20 | 19 | 21 | 20 |
| Female | 60 | 57.1 | 59 | 56.1 |
| Total | 80 | 76.1 | 80 | 76.1 |
| P-value | -- | 0.0001 ** | -- | 0.0001 ** |
| ** (P≤0.01). | | | | |

There was highly significant differences (P≤0.01) according to the sex by both ELISA IgG and miniVIDAD IgG test.

**Table 11: The distribution of toxoplasmosis by ELISA-IgG and miniVIDASIgG (Ab) in females group in two regions in Baghdad province**

| Test / Females Group | | | | |
|---|---|---|---|---|
| | ELISA + | | miniVIDAS + | |
| | NO | % | NO | % |
| Pre-marital | 52 | 49.4 | 49 | 46.6 |
| Married | 8 | 7.6 | 10 | 9.5 |
| Total | 60 | 57 | 59 | 56.1 |
| P-value | -- | 0.0001 ** | -- | 0.0001 ** |
| ** (P≤0.01). | | | | |

There was highly significant differences (P≤0.01) according to the females groups by both ELISA IgG and miniVIDAD IgG test.

**Table 12: The seroprevalence of toxoplasmosis by ELISA-IgG and miniVIDAS-IgG (Ab) according to the female group (aborted and normal) between the two regions in Baghdad province**

| Test / Female Group | ELISA-IgG and miniVIDAS- IgG (Ab) | | | |
|---|---|---|---|---|
| | ELISA + | | miniVIDAS + | |
| | NO | % | NO | % |
| Aborted | 5 | 62.5 | 6 | 60 |
| Normal | 3 | 37.5 | 4 | 40 |
| Total | 8 | 100 | 10 | 100 |
| P-value | -- | 0.479 NS | -- | 0.527 NS |
| NS: Non-Significant. | | | | |

Non Significant differences according to the females groups (aborted and normal) in both test ELISA IgG and miniVIDAD IgG.

**Statistical Analysis:**

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of difference groups/ factors in study parameters. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability in this study.

**Note**:
* means significant (P≤0.05).
** means highly significant (P≤0.01).
NS means not significant.

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