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The Importance of Conducting a Toxoplasmosis Tests for Individuals and the Most Important of These Tests

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ABSTRACT

It is necessary to conduct a toxoplasmosis test for people of both sexes, especially blood donors, as this disease has different paths. The danger of the disease lies in the fact that it is transmitted in different ways and its symptoms do not appear in people with good immunity. It is only transmitted and its symptoms and complications appear in people with weak immunity. Toxoplasma gondii is a parasite that is widely spread in most Iraqi cities. This study demonstrated the necessity of screening blood donors for Toxoplasma gondii, as the parasite can be transmitted through the bloodstream, causing harm and complications. After examining the presence of the parasite, significant levels of it were found in the blood. Toxoplasma gondii was diagnosed using various diagnostic methods, the most common of which were rapid testing, molecular testing, and immunological testing. The cases were reported among blood donors of different ages and genders, especially those coming to blood banks in cities.

Keywords

Kufa, Parasite, *Toxoplasma gondii,* Toxoplasmosis test, Iraq.

Introduction

Toxoplasmosis is caused by the parasite Toxoplasma gondii. This disease is common among humans and may be present without symptoms. Toxoplasmosis is widespread worldwide, particularly among herbivores, carnivores, and omnivores, and affects all mammals and birds. The life cycle of this protozoan includes cats, small mammals, and birds [1]. It causes miscarriage in pregnant women, and if the fetus develops, it may later develop mental retardation, which is common [2].

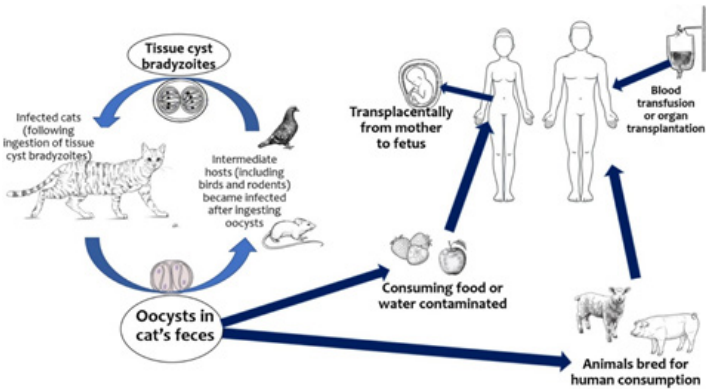
Chemotherapy is the main treatment, but current treatments are limited in use due to side effects and contraindications [3]. Latent toxoplasmosis has been found to affect certain semen parameters in men specifically sperm count and motility, but not sperm shape or size. Tobacco smoking has been found to exacerbate the negative effects of Toxoplasma gondii infection on semen parameters [4]. Toxoplasmosis ranges from asymptomatic disease in immunocompetent individuals to severe disease in immunocompromised individuals [5].

Toxoplasma gondii is a protozoan parasite that must live inside host cells to survive. As a unicellular eukaryote, it belongs to the Apicomplexa phylum [6]. Toxoplasma gondii is an intracellular protozoan that causes toxoplasmosis. It accounts for 25.7% of all infections. Transmission occurs through contaminated food and water containing the sporangia, or by consuming undercooked or contaminated meat. Other less common modes of transmission include congenital transmission, blood transfusion, and organ transplantation [7].

Stages of Toxoplasma gondii

There are three stages of T. gondii [8]: -

1. Oocysts, which give rise to sporozoites.
2. Tachyzoite or trophozoite, the actively proliferating form.
3. Cystozoite or bradyzoite, the resting form [9].



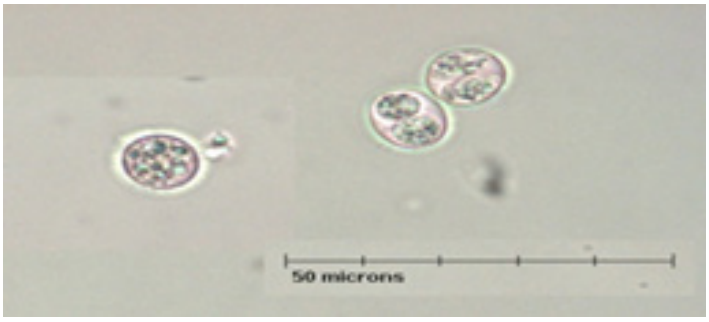
Life cycle of Toxoplasma gondii [10].

Toxoplasma gondii tests

There are groups of individuals for whom diagnosis of toxoplasmosis is of greater importance: pregnant women who become infected during pregnancy, fetuses and newborns with congenital infection, those with immunodeficiency, those with chorioretinitis, and blood donors to others [11,12].

Microscopic diagnosis

Microscopic identification of *Toxoplasma gondii* tachyzoites or bradyzoites viacytology or histopathology [13]. Under microscopic examination, the sporangia appear oval in shape, and each sac consists of two sporangia, each containing a spore with 4 zoospores [14]. Tissues obtained by biopsy or microdissection are examined directly by fixing, sectioning, and staining, or by making impression smears treated with multicolored dyes [9].



***Toxoplasma gondii*; Sporulated and Unsporulated Oocysts.**

Rapid diagnostic tests (RDTs)

Rapid diagnostic tests (RDTs), especially those with point-of-care (POC) features, are excellent diagnostic methods in clinical microbiology laboratories, especially in areas with limited laboratory facilities [14]. Two milliliters of the patient's blood are collected by venipuncture and placed in EDTA tubes. The sample is then centrifuged at 1780 × g for 10 minutes at 40°C to separate the plasma. The plasma is then tested for IgG and IgM antibodies to T. gondii using immunochromatography (ICT) testing. This ICT technology is a rapid, one-step TOXO IgG/IgM test WB/S/P. It is a lateral flow chromatographic immunoassay for the simultaneous detection and differentiation of IgG and IgM antibodies to *Toxoplasma gondii* in plasma or whole blood. The testing procedures are followed strictly according to the manufacturer's instructions [15]. Rapid parasite Testing is easy and quick to perform, and does not require trained personnel or special equipment. However, rapid parasite testing has been reported to be unreliable in diagnosing *Toxoplasma gondii* [16]. Recent evidence suggests that rapid immunochro-matographic tests (ICTs) could be used as an alternative to laboratory techniques and provide rapid results. ICTs employ a combination of monoclonal antibodies conjugated with colloidal gold particles and recombinant *T. gondii* anti-gens bound to the solid phase of a nitrocellulose membrane to detectanti-Toxoplasma antibodies in feline whole blood, plasma, and serum [18]. The CMIA method Chemiluminescent microparticle immunological assay is commercially available for research use for *T. gondii* IgG measurements in many countries, including Japan, where it has been used to detect maternal *T. gondii* infection during early pregnancy or the risk of congenital *T. gondii* infection [19].

Rapid diagnostic tests

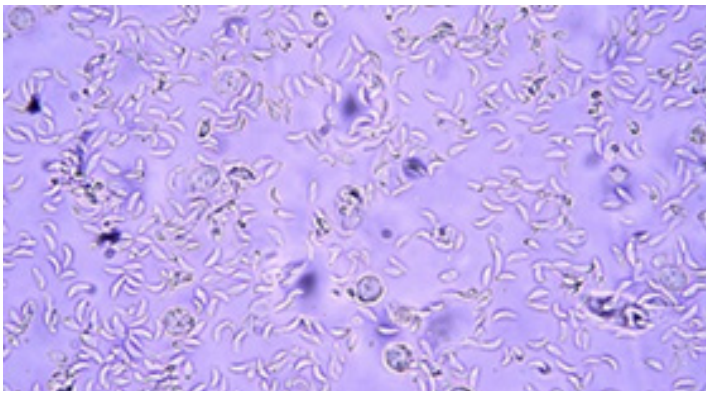
Serological tests

Toxoplasma gondii infection often causes no clinical symptoms or nonspecific symptoms in most individuals, whose diagnosis is based primarily on serological tests. Serological techniques are easy to use and cost-effective, facilitating the monitoring of *T. gondii* infection, several serological techniques have been developed for the detection of *Toxoplasma gondii* infection over the past decade [19]. The predominant technique for diagnosing toxoplasmosis is serological testing for toxoplasmosis-specific immunoglobulin G (IgG) antibodies. Levels of toxoplasmosis-specific immunoglobulin M (IgM) antibodies rise after a period of infection, but they typically decline rapidly and become undetectable within weeks or months. IgG levels rise within one to two months after infection and remain elevated throughout life. The presence of toxoplasmosis-specific IgG antibodies indicates current toxoplasmosis infection, while IgM antibodies indicate recent infection. Serum toxoplasmosis antibody levels are measured using several methods [9].

Dye test(DT)

The Sabin-Feldman test, or staining test (DT), is a traditional diagnostic method developed over 70 years ago and performed in laboratory settings. It is the most common and still in use. Live

parasites are incubated with a serum sample and stained with methylene blue. The principle is that the neutralizing antibodies to *Toxoplasma gondii* and the complement factors in the serum damage the outer structure of the live parasites, preventing staining. The antibody standard test involves calculating the ratio of live to dead parasites, or the serum standard as the highest dilution at which the sample will be positive. Because it relies on live parasites, this method is performed in laboratories and requires skilled technicians. False negative or false positive results are possible [20]. Live Toxoplasma are treated with a complement-like factor and examined in serum at 37°C for 1 hour before adding methylene blue. The presence of a membrane-permeable antibody in the parasite allows cytoplasmic leakage, which renders Toxoplasma unable to absorb the dye and, therefore, appears colorless. Toxoplasma not exposed to a specific antibody (i.e., a negative serum sample) absorbs the dye and exhibits a blue color. This DT test is an accurate and sensitive serological test for humans, but is unreliable for other species [9].

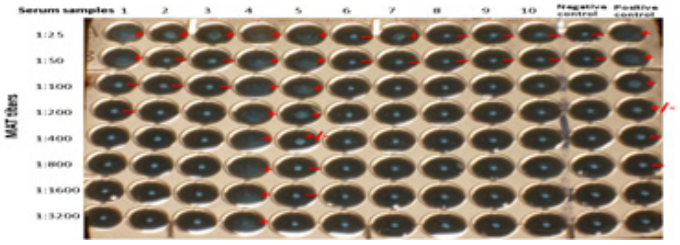


The sabin-Feldman dye showing a positive result for Toxoplasmosis.

The agglutination test

Modified agglutination test (MAT)

One test that has both sensitivity and specificity is the modified aggregation test (MAT) using preserved whole tachyzoites [21]. The modified aggregation test (MAT) detects specific antibodies in various body fluids, especially serum. The MAT is simple, easy to use, and accurate [22]. The modified agglutination test (MAT) is widely used to detect antibodies to T. gondii and is considered a safe, effective, and highly sensitive method for identifying previous infections. This serological test is designed to detect IgG antibodies in mammals, including humans, and in birds [23].



Direct agglutination test (DAT)

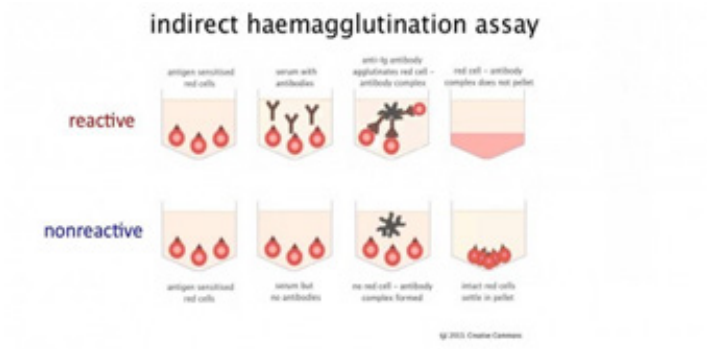
Latex agglutination test (LAT)

This test is used in many scientific studies to detect toxoplasmosis in patients' serum [24]. The test is performed using the Toxo Latex Kit. Designed for qualitative and semi-quantitative testing, the test is performed according to the company's instructions. For interpretation of results, the highest serum dilution showing agglutination corresponds to the *T. gondii* antibody titer [25].

Toxo Latex Kit

Indirect hemagglutination test (IHAT)

The reagent consists of a suspension of fixed red blood cells coated with purified antigen derived from the parasite *Toxoplasma gondii*, incubated in mouse peritoneal secretions. These cells react with specific antibodies present in human or animal serum, forming a uniform network on the plate (a positive reaction). If specific antibodies are present, the red blood cells aggregate in a characteristic button at the bottom of the plate, indicating a negative reaction. This method is applicable to both humans and animals and is efficient in testing large quantities of serum. However, it is not suitable for detecting congenital or neonatal infections and is susceptible to variations in red blood cell quality and antigens [9].



Immunosorbent agglutination assay (ISAGA)

The ISAGA immunocapture test for the detection of anti-Toxoplasma Immunoglobulin M is a manual technique known for its excellent sensitivity and specificity [26]. Comparative performance of ISAGA IgM and ELISA assays for the diagnosis of maternal and congenital Toxoplasma infections: which technique could replace ISAGA IgM?. *Parasite*, *31*, 7.. the IgM immunosorbent agglutination assay (ISAGA) is a help to

determine whether the IgM are specific or not [27]. Due to changes in European regulations for in vitro diagnostics, production and marketing of ISAGA will be discontinued in 2024. The IgM ISAGA test is considered to be the method of choice for the detection of IgM in infants <6 months of age [26].

Indirect fluorescent antibody test (IFAT)

The indirect immunofluorescence assay (IFAT) is a microscopic test that uses parasites mounted on a slide. Diluted patient serum is added, and if antibodies specific to toxoplasmosis are present, they bind to the parasites and are detected by a fluorescent antibody against the μ-chain. While IFAT has negative aspects, interpreting the fluorescence is a complex process requiring expertise, resulting in inconsistent results. Low levels of IgM can be difficult to distinguish, and cellular remnants can cause confusion. False-positive results for the IFAT IgM test are possible, and high concentrations of IgG in the sample can competitively inhibit IgM binding, leading to false-negative results [9,28].

Enzyme-linked immunosorbent assays (Elisa)

The most reliable diagnostic method for toxoplasmosis is to detect the pathogen, although it is expensive, time-consuming, and less sensitive. The ELISA test for detecting Toxoplasma plasma antibodies has been found to have high sensitivity (92%) and high specificity (91%) compared to the indirect immunofluorescent antibody test (IFAT), which has been used as the reference test [29].

Avidity test

The avidity of *Toxoplasma gondii*-specific IgG antibodies is an important tool for distinguishing recent from recent infections. The IgG avidity test is a serological indicator of toxoplasmosis, in many cases confirming or excluding the active form of the disease. IgG antibodies produced during a recent primary *Toxoplasma gondii* infection are characterized by low avidity, whereas IgG antibodies with high avidity are detected in the chronic phase of infection. There are important areas of current research related to the use of recombinant parasite antigens, which may improve the performance of IgG avidity tests [9,30].

Imaging techniques

CT scan remains useful in the evaluation of focal brain lesions and facilitates the diagnosis of cerebral toxoplasmosis, especially in conditions of limited access to MRI. CT significantly underestimates the number of lesions later detected in post-mortem examination. The authors suggest that CT scans allow demonstration of the progression of cerebral toxoplasmosis and evaluation of the effectiveness of treatment [31]. Imaging methods such as positron emission tomography (PET), positron emission tomography with computed tomography (PET/CT), and single photon emission computed tomography (SPECT) can be useful in the diagnosis of toxoplasmosis of the central nervous system [32].

Molecular technologies

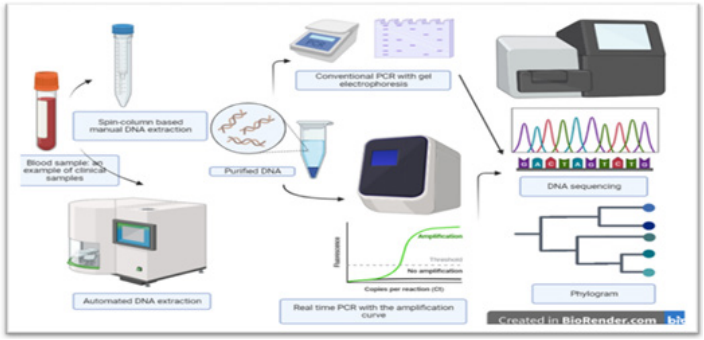
Molecular techniques such as polymerase chain reaction (PCR) use blood serum and samples from which DNA and messenger RNA can be extracted, such as amniotic fluid, blood, brain tissue, eyes, and urine [20].

Polymerase chain reaction (PCR)-based molecular techniques

With the new diagnostic methods of polymerase chain reaction (PCR), it has become possible to diagnose *Toxoplasma gondii* infection using molecular techniques [20]. PCR is a good method for detecting the disease, but it does not differentiate between the current pathogenic process and the past infection [31].

Real-time PCR

Real-time polymerase chain reaction (PCR) is a molecular diagnostic method that uses the principle of gene amplification toxoplasmosis. This reaction detects infection at very low concentrations and can determine the number of *Toxoplasma gondii* parasites in tissue samples that cannot be measured by serological analysis. This reaction is characterized by high sensitivity and speed, and it does not require parasite isolation [20]. Conventional PCR was set up for confirmation of positive samples [33].



Real-time PCR

PCR-RFLP

PCR-RFLP for identification genotype of the parasite, PCR-RFLP method using genes was applied and fragments of DNA [34]. A simple protocol was developed to convert DNA sequences into RFLP genotyping data in the parasite *Toxoplasma gondii*. This method could potentially combine traditional PCR-RFLP genotyping with whole genome sequence data, facilitating long-term population genetic studies [35].

Microsatellite analysis

MS sequences, in their various forms, are found in the genomes of all living organisms. When these sequences are amplified, either single or multiple polymerase chain reaction (PCR) is used, employing pairs of primers. One primer in each pair is labeled with a fluorescent dye. The amplification products are then separated using a filament sequencing device, allowing for the determination of the lengths of the MS fragments [36]. Microsatellites are genetic markers. They are short, tandem repeats of 2–6 nucleotides. The markers generated by these repeats are known to be highly polymorphic due to their varying lengths. Therefore, they exhibit multiple alleles, making them extremely useful for genetic studies.

Polymorphism is assessed using polymerase chain reaction (PCR), which requires a small amount of DNA. Allele size can be determined using fluorescent primers and an automated sequencer, ensuring reliable results Multi-locus genotyping with multiple microsatellite markers on Toxoplasma gondii parasite samples was used to design a molecular tool [37].

Western blotting (WB)

This assay is used to identify specific immunoglobulin bands present in crude antigens of RH and rapid strains isolated from T. gondii [38]. The amount of T. gondii proteins used in IgG-WB was determined using the method of Lowry, using a 12% polyacrylamide gel for protein electrophoresis with subsequent transfer of the proteins to a nitrocellulose membrane [39].

Multilocus sequence typing (MLST)

Multi-locus sequence typing (MLST) of both highly conserved and more polymorphic areas of the genome, and Multi-locus sequence typing (MLST) provides several advantages, including accuracy and specificity, over conventional approaches [40]. There are several genetic classifications of Toxoplasma gondii strains. The original classification was a clonal group with three lineages, namely types I, II, and III, and the so-called "a typical" strains, which have great genetic diversity. The classifications evolved by dividing the strains into six branches and 16 haplogroups based on multi-locus sequence typing (MLST) and other patterns [41].

Loop-mediated isothermal amplification (LAMP)

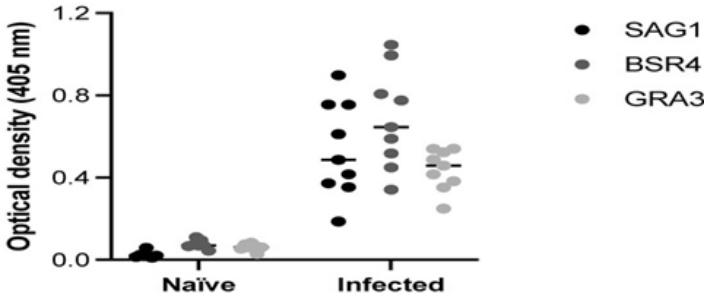
LAMP was more accurate than polymerase chain reaction (PCR) in detecting toxoplasmosis infection in mice, detecting infection in blood as early as the first week after infection [42]. LAMP is a nucleic acid amplification test used in various fields, including infection diagnosis, to identify organisms. This test uses a DNA polymerase called Bst polymerase, which has a translocation activity, and four primers specifically designed to recognize six distinct sequences of target DNA [43].

High-resolution melting (HRM)

High-resolution melting analysis is a rapid and effective diagnostic tool. HRM analysis has been used to identify and differentiate *Toxoplasma gondii* genes in numerous scientific studies [44]. A new method for genotype determination has been developed using high-resolution melting curve (HRM) analysis. The first of this technique was using the 35-fold repeat B1 gene. Some HRM profiles enable genotype determination [45].

Serotyping methods based on polymorphic polypeptides

Serotyping is a method for identifying *Toxoplasma gondii* strains that does not require parasite isolation. Serotyping is based on the detection of antibodies to polymorphic peptides specific to each strain. Various strategies have been described for serotyping *Toxoplasma gondii*. [46]. The life cycle of *Toxoplasma gondii* is complex, and the expression of its proteins changes during different stages of development. Some of these proteins have been shown to activate the immune system and generate antibody levels that persist throughout the host's life [47]. The surface antigens SAG1 and SAG2A found in the *Toxoplasma gondii* parasite are highly immunogenic proteins. SAG1 has been used in serological studies that have shown its effectiveness in detecting IgG antibodies in individuals with chronic infection [48].



The ELISA test used SAG1, BSR4 and GRA3 peptides to act as antigens [48].

Conclusion

Toxoplasmosis is a disease that is noticeably widespread in Iraq in particular, and according to many scientific studies, it is one of the diseases that have serious complications, especially since its symptoms are similar to other diseases. This disease may not come to mind, and it is necessary to conduct an examination to detect the disease in order to treat it early and to avoid transmitting it to other individuals through the methods by which it is transferred, especially blood donors to people in need of blood. Blood must be tested with the toxoplasmosis test available in the country before it is transferred, as its transfer results in dire consequences that affect men, women and children alike. Currently, in Iraq, in most hospitals, there are no tests for this disease, perhaps due to the costs or the absence of people with expertise in the field of parasite examination. However, tests are available in external non-governmental laboratories, where they are conducted for people suspected of being infected under the guidance of a specialist doctor, and the examination is usually microscopic. It is possible to test for toxoplasmosis with a rapid test as a first step with tests from reliable companies and then confirm it by any other testing method.

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