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# Diagnostic Modalities of Toxoplasmosis

**Amany Abdel Rahman Mohamed Maghawry, Afaf Abd El-Raouf Taha, Dalia Abd El-Khalik Abo El-Maaty, Amira Abd El-Lateef saleh**

Medical Parasitology Department, Faculty of Medicine, Zagazig University, Egypt.

**Email:** amanyofa4122015@gmail.com, Aammoghawri@medicine.zu.edu.eg

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**Abstract:** Toxoplasma gondii was first isolated from a common gundi (Ctenodactylus gundi) in Tunis in 1908 and the same year in a rabbit from South America. Six clades have been characterized using population genetic structure studies indicating that globally diverse isolates originate from a small number of ancestral lineages .The diagnosis is critical for the surveillance, prevention and control of toxoplasmosis. Traditional approaches to the laboratorial diagnosis involve etiological, histopathological, immunological, immunohistochemical and molecular methodologies. Recently, two biological approaches are used: (i) direct detection as parasitological methods using microscopy to parasite detection and (ii) indirect detection through immunological assays. The other is the molecular methodology that detects the parasite DNA in suspected samples.

**Keywords:** Toxoplasmosis

## Introduction

*Toxoplasma gondii* was first isolated from a common gundi (*Ctenodactylus gundi*) in Tunis in 1908 and the same year in a rabbit from South America. Six clades have been characterized using population genetic structure studies indicating that globally diverse isolates originate from a small number of ancestral lineages **(1)**.

The name *Toxo* means 'arc form' in Greek and was named according to the crescent-shaped morphology of the tachyzoite and bradyzoite stages of the organism; plasma for life and *gondii* for the host where it was first found **(2).**

During the first decades of the twentieth century, the existence of several species of the genus *Toxoplasma* infecting different hosts was considered **(3).**

The first human case ascribed to infection with *T. gondii* was a child with hydrocephalus; also **Sabin in (4)** reported the first case of encephalitis due to *T. gondii.* During the 1940s, there was an improved understanding of the cause of maternal infection for congenital toxoplasmosis in newborns. It was Reported reported a "series of 103 children, 99% of whom had eye lesions, 63% had intracranial calcifications and 56% had psychomotor retardation**."**

The parasite was found to be capable of infecting all warm- blooded animals including human. The pathogenic potential of *T. gondii* was recognized in 1920s and 1930s **(2).**

The manifestations of toxoplasmosis vary among patients. This pathologically classified into acute, chronic, congenital, ocular infection and reactivated chronic toxoplasmosis **(5)**.

**Toxoplasmosis in Immunocompetent Patients :**

**Acute Toxoplasmosis:**

Acute toxoplasmosis progresses after an incubation period of a few days following tachyzoites' spread and replication. It is asymptomatic in more than 80% of immunocompetent individuals.However; severe cases have also been reported in immunocompetent persons. It can manifest with flu-like symptoms including fever and mononucleosis-like symptoms, with posterior cervical adenopathy, myalgia, and asthenia **(6).**

**Peng *et al.,* (7)** reported that infected lymph nodes are tender, palpable but not painful and the symptoms resolve in weeks or months. Lymphadenitis and lymphadenopathy are often accompanied with fever, malaise, fatigue, muscle pain, sore throat and headache. Postnatally acquired infections of *T. gondii* can also infect the eyes resulting in retinochoroiditis.

When the patient acquires immunity symptoms recede and the individual is protected for life time against re-infection **(7).**

**Congenital Toxoplasmosis:**

In sero-negative pregnant women, primary infection with *T. gondii* occurs following the placental transmission of the parasite to the fetus**.** The degree of severity of congenital toxoplasmosis is inversely associated to the gestational trimester at which the infection is acquired **(8).**

However the placenta represents a major forefront that inhibits tachyzoites' transmission in the beginning of gestation, this ability decreases gradually throughout the pregnancy, allowing the tachyzoites to move between cells and infect the fetus**.** It was assessed that about 25% of *T. gondii* transmission takes place in the first trimester, whereas 54% and 65% of transmission occur in the second and third trimesters, respectively **(9).**

Transmission to fetus in the first trimester often leads to abortion, stillbirth, or a child born with severe abnormalities of the brain and eyes, such as hydrocephalus, intracranial calcifications, deafness, mental retardation, seizures, retinochoroiditis, and even blindness**.** Infection of the mother to the fetus during the second or third trimester is less likely to cause such severe clinical manifestations, but may result in subclinical disease, which may lead to learning difficulties or retinochoroiditis after birth **(2).**

It is worth observing that the percentage of acquiring toxoplasmosis during pregnancy differs according to regions and prevalence and re-infection with atypical *T. gondii* genotypes was reported even in sero-positive pregnant women and lead to severe congenital toxoplasmosis **(10).**

**Ocular Toxoplasmosis:**

**Vallochi *et al., (11)*** reported that *T. gondii* is one of the primary causes of infectious uveitis worldwide, typically presenting with retinochoroiditis. Ocular toxoplasmosis commonly occurs after an acquired congenital toxoplasmosis. Some studies reveal postnatal acquired infections leading to this manifestation **(12).** Clinical features of ocular toxoplasmosis related to the anatomical location of the lesion **(12).** It was said that typically, retinochoroiditis is the most major indication of active intraocular inflammation. It presents with vitritis, posterior uveitis, focal necrotizing granulomatous retinitis, and reactive granulomatous choroiditis**.** The rupture of intra-retinal cysts may lead to the reactivation of ocular toxoplasmosis, enhancing a rapid localized immune reaction involving mostly Interleukin-17A **(13).**

**Chronic Toxoplasmosis:**

**Schlüter and Barragan, *(14)*** stated that *T. gondii* can be classified as a primarily neurotropic pathogen, having a higher affinity for the central nervous system over other organs. Direct symptoms of chronic toxoplasmosis are not fully revealed, and most available studies associate this disease status with neuropathies with only little molecular proof**.** However, the reactivation of chronic toxoplasmosis after immunosuppression is commonly reported and may lead to dire consequences reaching death **(5)**.

**1- Toxoplasmosis in Immunocompromised Patients:**

It was reported that with the growing number of individuals receiving immune-suppressive therapies, clinicians are aware of the potential occurrence of *Toxoplasma* encephalitis, not only during the reactivation of

latent infection, but also as a primary infection. Indeed, even though the availability of prophylactic and treatment options, the reactivation of chronic toxoplasmosis still occurs and can become life threatening **(13).** Toxoplasmic encephalitis is the predominant manifestation of the disease in HIV patients, while disseminated or pulmonary toxoplasmosis is more characteristic of transplant patients**.** These patients present with neurologic symptoms, most commonly diffuse encephalopathy, cerebral mass lesions, meningoencephalitis, headaches, confusion, seizures and poor coordination. Additionally, it was reported that there was a relationship between CD4 counts and the prevalence of *T. gondii*-related neurologic symptoms in patients with HIV. In that sense, the reactivation of chronic toxoplasmosis occurs when the CD4 count decreases below 200 cells / microliter.

This reactivation is due to the consequential reduction in IFN-γ and cytokine production, leading to impaired cytotoxic T-lymphocyte activity. Recent data revealed that HIV patients who presented with symptoms of dizziness and fever as part of their Toxoplasmic encephalitis prodrome sought medical care earlier than those who did not present with these symptoms, leading to the rapid administration of treatment, thus reducing mortality **(15).**

A retrospective review of solid organ transplant and hematopoietic stem cell transplant recipients with toxoplasmosis among 2002 and 2018 at two large US academic transplant centers was recently showed. The median time from transplant to toxoplasmosis diagnosis was longer for solid organ transplants than for hematopoietic stem cell transplants, and clinical manifestations were encephalitis (65%), respiratory failure (40%), renal failure (40%), and distributive shock (40%). The cohort 30-day mortality was 45%, and the 90-day mortality was 55% of the cohort **(16).**

**2- *Toxoplasma gondii* Associated Diseases:**

It was reported that in healthy individuals, chronic toxoplasmosis was considered as clinically asymptomatic. Though, an increasing number of associations are being made among *T. gondii* infections and various medical. These include primary neuropathies, behavioral and psychiatric disorders, and various types of cancer **(17)**.

**Diagnosis of toxoplasmosis:**

The diagnosis is critical for the surveillance, prevention and control of toxoplasmosis. Traditional approaches to the laboratorial diagnosis involve etiological, histopathological, immunological, immunohistochemical and molecular methodologies. Recently, two biological approaches are used: (i) direct detection as parasitological methods using microscopy to parasite detection and (ii) indirect detection through immunological assays. The other is the molecular methodology that detects the parasite DNA in suspected samples **(18).**

**Direct *T. gondii* Detection:**

**1- Parasitological diagnosis:**

*T. gondii* detection in water, environmental and tissue samples such as suspicious blood samples, cerebrospinal fluid, and lymph node or other corporal fluids or tissues in the peritoneal cavity of the immunosuppressed mice has been traditionally done using microscopy. Though, the identification depended on this method is unreliable and less sensitive than other methods. The parasite can be previously detected after fixing the specimen with methanol and staining with Wright or Giemsa stain. The parasites appear with dyed blue cytoplasm and a red nucleus at the center of the cytoplasm Giemsa and Hematoxylin/Eosin (HÆ) staining are simple and economical and commonly used for this purpose**.** The detection of tachyzoites in the peritoneal fluid of the mice is done by phase contrast microscopy from 6 to 10 days after inoculation. Electron microscopy is also used to detect tissue cysts in the brain of mice and oocysts in the small intestine of infected cats, but it is difficult to apply for routine use **(19).**

**2- Histopathological studies:**

Identifying of histopathological changes during some parasitic invasions is mainly important for differential diagnosis and often confirms the presence of parasitic diseases **(20).**

It was found that presence of tachyzoites in histological sections from suspicious biopsies indicates an acute infection. In contrast, the detection of tissue cysts that contain bradyzoites in histological samples only confirms a chronic toxoplasmosis infection. The detection of cysts in the placenta, foetus or newborn indicates a

congenital infection. According to the number of cysts in histological sections active infection that requires immediate treatment can be recommended.

The encysted tachyzoites and bradyzoites are usually demonstrable with Wright, hematoxylin and eosin dyes in biopsy or autopsy material. There are other stain techniques like Schiff-periodic acid, specific immunohistochemistry stained with immunoperoxidase that are equally efficient **(21).**

**Indirect Methodologies:**

Immunological and imunohistochemical diagnosis a wide range of serological tests for *T. gondii* detection is available with various methodologies and antigens that can detect different immunoglobulin isotypes. Most are not Rapid Diagnostic Test (RDT) and are aimed at laboratories with good infrastructure and qualified personnel. Immunological techniques are relatively inexpensive, require a small sample volume, and can be used on live animals **(22).**

1. **Serological tests:**

Humans present a lifelong persistence of anti-*T.gondii* IgG antibodies and, in many geographical areas, toxoplasmosis prevalence is high. Thus, in many cases, the serological titers do not reflect a recent infection. In this way, it is often the lack of reliability in discriminating recent from old infection by detection of anti-*T. gondii* IgM, IgA, or IgE. This situation is very common in congenital toxoplasmosis diagnosis **(23).**

IgM antibodies are detectable about 1 week after the infection and remain for several months or years. So the detection of IgM antibodies alone is insufficient for the establishment of acute infection. IgA antibodies are considered to be a marker of acute infection, which are produced earlier than IgM, and may persist for several months. The shorter period of IgE may give a greater indication of current infection. While the presence of IgG antibodies suggests the occurrence of infection, but does not provide any information about the timing of infection **(24).**

In the case of cerebral toxoplasmosis, the identification of a positive serology is less useful in settings where the seroprevalence for *T. gondii* in the general population is very high. In Brazil, most AIDS patients with any focal brain lesion also show positive serological diagnosis for toxoplasmosis. However, the identification of a negative serological result presents a high negative predictive value **(23).**

**A. Modified agglutination test (MAT):**

MAT is a sensitive serological method to detect *T. gondii* IgG antibodies in herds and wild animals. MAT primarily detects antibodies from animal tissue fluid, serum or plasma**.** It is the most widely used method for diagnosis, the most economical and the simplest among many methods available to detect *T. gondii* infection, and there is no need for specific equipment. In most laboratories, the development of MAT is performed with the aid of tachyzoites collected by traditional propagation performed in murine peritoneal cavity *in vitro* culture**.** Among the advantages of this method is that the antigen is stable at 4◦ C for many months and does not need species-specific reagents **(25).**

**B. Enzyme-linked immunosorbent assay (ELISA):**

ELISA has been used as the most reliable, practical, economical and widely used for detecting exposure to *T. gondii* in hosts**.** Only a small sample volume is needed, and the assay can be semi-automatic, making it suitable for large-scale screening. Additionally, different ELISAs can discriminate among immunoglobulin classes. Therefore, it is useful for determining the infection stage**.** Different methodologies for ELISA were standardized using various antigens (native, recombinant, chimeric), secondary antibodies and antibody binding reagents **(26).**

However, the choice of components can significantly influence test performance. Commercial ELISA kits for the detection of *T. gondii* antibodies in domesticated animals are available, making large-scale and routine screening feasible **(18).**

**Avidity tests:**

The IgG avidity test was described by **Hedman *et al.,* (27).** This assay measures the avidity of the binding of specific antibodies to *T. gondii* antigen. The proteins present in the serum are denatured with a solution of urea. The avidity can be variable during the course of infection**.** In early stages of infection, the values of avidity are low and are increasing with the course of infection **(27).** Therefore, the avidity test can distinguish between acute and chronic infection **(28).**

Avidity testing is mostly performed in combination with ELISA and can be used for IgG, IgA and IgE. Although useful as an indicator of a recent infection, anti-toxoplasmic low-avidity IgG can persist during the entire pregnancy. Treatment with spiramycin could delay the avidity maturation **(28).**

**C. Indirect fluorescent antibody test (IFAT):**

IFAT is a simple test that detects IgG and IgM antibodies and has been widely used in the detection of antibodies against *T. gondii* in humans and animals. Fluorescence-labeled antibodies to a variety of species are commercially available. However, a fluorescence microscope is required for the test, the results are read with the naked eye, and individual variation may occur. Some species-specific conjugates can be difficult to be found, and there is a risk of possible cross-reactivity with rheumatoid factor and antinuclear antibodies **(29).**

**D. Sabin Feldman Test Dye test (DT):-**

DT was first developed by **Sabin and Feldman, (4).** It has been considered a gold standard test for the detection of anti-*T. gondii* antibodies in humans**.** However DT is specific and sensitive in humans, it may be unreliable in cattle and avian species **(30).**

It has disadvantage as it requires live parasites and healthy human serum as an accessory factor, which severely limits the availability of the DT**.** The test is potentially hazardous, and requires a high degree of technical expertise, thus only performed in reference laboratories. Though tachyzoites prepared from cell culture can be routinely used in DT, the false negative results may occur in some cases. Therefore, tachyzoites prepared from mice are preferred for DT **(31).**

**2. Immunohistochemical diagnosis:**

IHC is an integration of histological, immunological and biochemical techniques, which is used for the identification of specific tissue components (antigens) by means of a specific antigen/antibody visible reaction. IHC visualize the distribution and localization of specific cellular markers or components within a cell or tissue **(32).**

The most widely used and highly sensitive immunohistochemical method is avidin-biotin method or commonly called avidin-biotin complex (ABC). The most commonly used avidin-biotin method is labeled avidin-biotin (LAB) or labeled streptavidin avidin-biotin (LSAB) with biotinylated secondary antibody and three reagents from peroxide or alkaline phosphate. This method has higher sensitivity compared to other ABC methods**.** Specific immuno-histochemical staining using anti-sera to *T. gondii* may demonstrate *T. gondii* antigen and tachyzoite in lesions. Most polyclonal antisera react much more strongly with tachyzoites than bradyzoites. Tissue cysts can be stained much weaker than tachyzoites in the same section **(7).**

**3. Molecular Diagnosis (Polymerase chain reaction (PCR) :**

According to molecular diagnosis, PCR is the methodology used to detect *T. gondii* DNA in clinical, veterinary and environment samples. It is considered highly specific and highly sensitive method**.** The test sensitivity can be influenced by different factors as heterogeneous distribution of bradyzoite cysts within tissues, variation in tissue predilection by *T. gondii* in host, tissue quantity and tissue selection **(33).**

It can be used in situations where sera are not available or antibody preservation is compromised, as, in wildlife studies when the eradicated carcasses are the most readily available set of samples. Additionally, PCR-based techniques can be expanded to permit for sequencing and typing of strains, which can be vital in detailed epidemiological studies **(18).**

In humans, PCR have allowed the sensitive detection of *T. gondii* DNA in clinical specimens such as amniotic fluid, cerebrospinal fluid (CSF), aqueous humor, blood, and bone marrow blood**.** PCR is suitable for AIDS patients, since this method is not influenced by the immunological status and has shown to be rapid, sensitive

and specific, avoiding expensive and invasive brain biopsy techniques. As an alternative approach, PCR in CSF and peripheral blood samples has also been used with numerous reported sensitivities**.** The development of the Real-time PCR (qPCR) has revolutionized molecular diagnosis by adding speed and reliability, representing significant progress**.** Many reports tend to generalize the idea that qPCR in general has an improved sensitivity compared with conventional PCR **(34).**

Subsequently, variations of PCR sensitivity and specificity relay also on factors including the standardization of reagents and protocols for DNA extraction, the time elapsing among the start of specific therapy and collection (CSF or blood) and the inadequate storage of the clinical sample which often affects PCR reproducibility and makes comparison of results difficult **(34).**

Several DNA targets for PCR were assessed and have been used regularly in the different laboratories to detect the diagnosis of congenital, ocular, disseminated or cerebral toxoplasmosis. Targets such as bradyzoite genes encoding for specific antigens, B1 gene; P30, ribosomal DNA genes and 529-bp sequence have been often used **(34)**, or to detect and quantify parasite DNA load and its levels in various moments of infection, as during the course of therapy **(35).**

**Imaging techniques:**

Imaging techniques, like magnetic resonance imaging (MRI), computed tomography (CT) and ultrasonography (US) are not specific tools. Though, they can simplify the diagnosis of toxoplasmosis and evaluate the therapeutic effect**.** Imaging using MRI or CT is vital for diagnosis of cerebral toxoplasmosis. Single or multiple focal lesions are usually found with CT or MRI, with a typical pattern of a hypodense lesion with perilesional edema **(23).**

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