Toxoplasmosis: Status, control strategies and prospective challenges

Review Article

Khloud K Mohsen, Marwa A Gouda, Ayman A Abdel-Wahab

Department of Clinical and Molecular Parasitology, National Liver Institute, Menoufia University, Menoufia, Egypt

ABSTRACT

Toxoplasmosis is a widespread disease infecting about one-third of the world's human population. Until now, a live-attenuated vaccine (Toxovax) is the only commercially available vaccine that has several limitations for use in humans, while the current treatment for toxoplasmosis has limited efficacy in eradicating the infection and is associated with several side effects. Therefore, the search for effective preventive and control strategies for toxoplasmosis is mandatory. This comprehensive review aims to provide the current and emerging strategies for control of toxoplasmosis. The study highlights different approaches to control toxoplasmosis that include public health education regarding the hazards of the parasite and precautions that should be taken to avoid infection; procedures generally done to ensure sanitary food and water supplies; preventive screening measures to prevent transmission of toxoplasmosis through organ transplantation and blood donation; advanced diagnostic techniques; emerging chemotherapeutic targets, and promising vaccines for toxoplasmosis.

Keywords: chemotherapy; control measures; diagnostic tools; drug targets; nanotechnology; toxoplasmosis; vaccination.

Received: 13 June, 2025; **Accepted:** 31 August, 2025.

Corresponding Author: Khloud K. Mohsen, **Tel.:** +20 1091148681, **E-mail:** drkhloud.kamel@liver.menofia.edu.eg

Print ISSN: 1687-7942, **Online ISSN:** 2090-2646, **Vol. 18, No. 2, August, 2025.**

INTRODUCTION

Toxoplasmosis is a prevalent disease caused by infection with the obligate intracellular parasite *T. gondii*, affecting nearly all warm-blooded animals and humans[1]. It is classified within the phylum Apicomplexa, distinguished by specialized apical secretory organelles known as micronemes and rhoptries, which are essential for the parasite's survival and propagation. Additionally, it possesses a third type of secretory organelle, the dense granules, which are located in the cytosol and discharge their contents to modify the parasitophorous vacuole and reprogram the host cell[2].

This apicomplexan is an opportunistic and effective coccidian parasite characterized by a complicated life cycle, capable of infecting almost all homeothermic animals, including humans. Domestic cats and other members of the Felidae family serve as the primary definitive hosts, whereas humans and other non-feline species are classified as intermediate hosts[3]. The oocyst infective stage is the most resilient form to environmental conditions and has significant resistance to disinfectants, which is crucial in the transfer of infection to humans[4]. Human infection may occur via various routes, including the consumption of undercooked infected meat harboring *T. gondii* cysts, ingestion of oocysts from contaminated hands, food, or water, organ transplantation or blood transfusion, transplacental transmission, and accidental inoculation of tachyzoites[5]. Despite the acknowledgement of *T. gondii* as a major foodborne pathogen and the clear role of the meat borne transmission pathway in human infections, there are currently no recommendations for controlling *T. gondii* in meat[6].

Water used in irrigation methods, lakes, rivers, coastal areas, beaches, and wastewater and groundwater may be polluted by environmentally resilient oocysts. Furthermore, oocysts retain viability in water for 18 m at 4°C after exposure to 2% sulfuric acid and can survive even with the use of chemical agents, such as sodium hypochlorite and chlorine[7]. Attempts at inactivation of *T. gondii* are successful against other biological stages of the parasite. Nonetheless, cleaning potable water and fresh food from oocysts necessitates attention since their tough walls are a strong obstacle to physical and chemical assaults[8].

Adaptive and innate immunity significantly contribute to defense against toxoplasmosis. An efficient immune response regulates parasite proliferation while preventing immunopathology; the interferon (IFN-γ) and interleukin (IL-12) axis are the primary immunological mechanisms that are accountable for parasite regulation[9].

Toxoplasmosis may exhibit a broad range of clinical symptoms. In immunocompetent individuals, acute primary toxoplasmosis is regarded as either asymptomatic or exhibiting mild symptoms, including

 DOI: 10.21608/puj.2025.393988.1301

fever and mononucleosis-like manifestations, with or without lymphadenopathy. Severe, fulminant, and potentially fatal toxoplasmosis has been extensively documented in congenitally infected individuals and immunocompromised patients with significant immune deficits[10]. Congenital toxoplasmosis (CT) arises when a maternal infection is acquired for the first time during pregnancy. In the parasitemic phase, *T. gondii* may traverse the placenta and infiltrate the fetal circulation, with the risk of fetal infection rising with gestational age[11].

Until now, a live-attenuated vaccine (Toxovax) based on *T. gondii* tachyzoites (S48 strain) is the only commercially available vaccine. It has been licensed for use against toxoplasmosis in sheep, but it is not effective in preventing tissue cyst formation and has several limitations for use in humans[12]. Given the primary transmission pathways of *T. gondii*, a critical and pressing need exists to provide an effective vaccine for toxoplasmosis[13]. The established gold standard therapy for toxoplasmosis (pyrimethamine and sulfadiazine) effectively manages the active phase of the illness. Nevertheless, no treatment is effective against the latent phase of infection, partly due to the sluggish and asynchronous proliferation of bradyzoites[14]. Furthermore, the predominant pharmacological agents for managing toxoplasmosis in the general populace and pregnant women are spiramycin (SPM) and azithromycin; yet their efficacy may be compromised by insufficient blood-brain barrier (BBB) penetration and poor bioavailability[15]. Therefore, the search for effective preventive and control strategies for toxoplasmosis is mandatory.

Magnitude of toxoplasmosis and its complications

Although toxoplasmosis is common worldwide, it usually remains asymptomatic. However, serious complications can arise in vulnerable groups such as fetuses, newborns, and immunocompromised patients[5]. Approximately one-third of the global population is estimated to be infected with *T. gondii*, with seroprevalence rates ranging from 10% to over 90%. Highest rates were observed in Africa, Southeast Asia, the Middle East, Central and Eastern Europe, and Latin America. Based on continental infection rates, data revealed different seroprevalence rates among AIDS patients across continents; *i.e.*, Asia (13.3–85.3%), Europe (40–76%), Africa (21.74–74.8%), and North America (7.3–26.5%)[16]. In their report, Egyptian reviewers[17] claimed that anti-*T. gondii* IgG antibodies were recorded varying between 3-42.5%, whereas among healthy blood donors, the seroprevalence ranged from 33.7% to 67.4%. In Nigeria, 26.8% seroprevalence among women of reproductive age was recorded[18]. In Iran, toxoplasmosis affected 62.2% of individuals across different population groups, with notable correlations observed between the infection, and risk factors such as age, contact with soil, and occupational exposure[19]. The estimated monthly prevalence of toxoplasmosis among pregnant individuals across Japan was 0.016% at the national level[20].

It is worth mentioning that *T. gondii* is one of the rare pathogens able to cross the placental barrier, with the risk of transmitting the infection to the fetus rising as pregnancy advances. Around 60% to 81% of infections take place during the final trimester; however, the consequences of infection are typically more severe when it occurs in the early stages of pregnancy[21]. Early CT may lead to spontaneous abortion. In contrast, infections acquired in the later stages of pregnancy are more likely to result in mild or subclinical disease. Of note, CT can cause a range of complications, including brain calcifications, hydrocephalus, cognitive and motor impairments, retinochoroiditis, and deficits in vision and hearing[22].

Additionally, *T. gondii* induces multiple changes in host neurons and disrupts specific neuronal signaling pathways during chronic infection. In fact, presence of the parasite within neurons leads to direct neuronal damage and a decline in neuronal function, including cell death and atrophy[23]. Chronic toxoplasmosis in the brain can lead to significant alterations in neuronal structure, neurochemical balance, and behavior. These changes were associated with higher rates of psychiatric conditions including schizophrenia, bipolar disorder, personality disorders, self-directed aggression, and suicide attempts[24].

Ocular toxoplasmosis is a major global cause of posterior uveitis and can result in serious complications that threaten vision, including retinal detachment, choroidal neovascularization, and glaucoma. These manifestations may occur due to either congenital infection or infection acquired postnatally[25]. While ocular toxoplasmosis in adults was once thought to be a reactivation of congenital infection, a review by Kalogeropoulos *et al.*[26] indicated that the majority of ocular cases are likely the result of postnatally acquired infections. These cases typically manifest as posterior uveitis, characterized by a unilateral chorioretinal lesion and vitritis, with more severe presentations commonly observed in immunocompromised individuals.

Primary preventive measures against toxoplasmosis

Precautions for prevention of toxoplasmosis, include thorough washing of hands after handling raw meat and before eating, avoiding the consumption of undercooked meat, cleaning surfaces and utensils after contact with raw foods, carefully washing fruits and vegetables, and wearing protective gloves while gardening outdoors[27]. Extreme heat or cold can cause *T. gondii* encysted in meat to perish. Therefore, the meat of any animal should be cooked to 67°C or cooled to -13°C before consumption[28].

Significant outbreaks of toxoplasmosis were associated with water contamination by oocysts[7] which exhibit resistance to commonly used chemical disinfection methods for maintaining sanitary water supplies, including strong acids, chlorine, detergents, ozone, and ultraviolet radiation. Consequently, the implementation of filtration systems is essential[29]. Heat therapies demonstrate rapid efficacy. Their application in treating low volumes of water can effectively inactivate the parasite, yet heating may induce undesirable organoleptic changes in vegetables. Other methodologies employed including radiation and pressure treatments, proved effective without altering the physicochemical characteristics of food. Consequently, these treatments may serve as effective alternatives for managing *T. gondii* and other parasites in vegetables[8].

Screening of toxoplasmosis among organ transplantation and blood donation

In solid organ transplant patients, prevention of toxoplasmosis requires multiple strategies, including serologic screening of both donors and recipients, chemoprophylaxis, and ongoing serological monitoring of patient's post-transplantation[30]. Molecular detection of *T. gondii* in blood and body fluids, along with histopathological examination of affected tissues, is recommended for identifying tachyzoites in the diagnosis of acute toxoplasmosis in solid organ transplant recipients[31]. In addition, immunocompromised patients, individuals receiving multiple blood transfusions, and pregnant women must be administered *T. gondii*-free blood. To prevent the risk of toxoplasmosis transmission, screening for *T. gondii* in blood as well as blood products should be incorporated into the pre-transfusion blood testing protocol[32].

The prevention of toxoplasmosis cannot be achieved through donor selection and serological screening methods, given the high seroprevalence of toxoplasmosis among blood donors and the absence of reliable, approved diagnostic tests for toxoplasmosis. Furthermore, the rejection of blood donations due to positive serology test results threatens blood availability, particularly in regions with a high prevalence of toxoplasmosis. The capacity of *T. gondii* to persist and proliferate within leukocytes suggests that leukoreduction filters could lessen the risk of toxoplasmosis[33].

Advanced diagnostic techniques

In fact, the only conventional commonly used methods are serological assays for detection of *T. gondii* antibodies. However, immunological assays utilizing *T. gondii* tachyzoite lysate antigens or specific target antigens are the most frequently used approach[34]. Sabin-Feldman dye test is considered the gold standard for diagnosis of toxoplasmosis[35]. Distinguishing between acute and chronic toxoplasmosis using commercially available laboratory tests remains challenging. However, the IgG avidity assay can differentiate between recent and past infections: low IgG avidity indicates a recent infection, while high avidity suggests that the infection likely occurred more than four months ago[36].

Molecular techniques and genotyping

Various PCR-based techniques were established for diagnosis of toxoplasmosis, utilizing different clinical samples such as amniotic fluid, blood, and cerebrospinal fluid. Both conventional PCR and real-time PCR (RT-PCR) are commonly employed to detect *T. gondii* DNA and determine strain genotypes[37]. Isothermal nucleic acid amplification technology (INAAT) is considered a promising tool for diagnosing toxoplasmosis, as it enables fast DNA amplification. Among the INAAT approaches, loop-mediated isothermal amplification (LAMP) and nucleic acid sequence-based amplification (NASBA) were developed for the rapid detection of *T. gondii*[35]. It was reported that LAMP method can rapidly amplify a few copies of genetic material to 109 copies with high efficiency, and specificity under isothermal conditions. Moreover, DNA amplification can be easily observed either by monitoring changes in turbidity or fluorescence, or through a loopamp real-time turbidimeter[38]. On the other hand, NASBA method enables proceeding of each reaction step when intermediate amplification becomes available throughout constant temperature without the need for thermocycler. Therefore, NASBA reaction is more efficient than other molecular methods that are limited to binary increases per cycle[39].

Recently, microRNAs (miRNAs) gained much attention as potential biochemical markers for various diseases. Their stability allows for reliable detection in plasma samples through quantitative real-time PCR (qRT-PCR)[40]. Serum miRNA mmu-miR-511-5p was investigated for its role in detecting toxoplasmosis and is suggested to be a highly sensitive biomarker, capable of identifying ME49 and RH strain infections as early as one week and three days, respectively[41].

Genotyping plays a crucial role in distinguishing different circulating strains, tracking the origin of infections during outbreaks, and identifying the strains linked to specific clinical presentations of the disease[42]. Using restriction fragment length polymorphism (RFLP), the majority of *T. gondii* isolates from human and animal samples were assigned to one of three clonal lineages (biotypes I, II, and III). Additionally, there is a growing focus on the biological differences that exist among these distinct genotypes[43].

Nanotechnology applied in diagnosis

Nanomaterials offer promising advantages for the rapid detection of *T. gondii* antibodies, antigens,

and DNA. Their high sensitivity and specificity make them valuable tools for the screening and diagnosis of different stages of toxoplasmosis[44]. In particular, gold nanoparticles (AuNPs) are particularly advantageous for rapid test development because of their distinct optical, magnetic, and chemical properties, combined with their favorable biocompatibility, low toxicity, and the ease with which they can be synthesized and functionalized[45]. Biosensors utilizing AuNPs have been designed for the precise detection of nucleic acids and proteins, including the identification of *T. gondii* antibodies. Due to their high sensitivity and specificity, these biosensors have the potential to serve as advanced diagnostic platforms, offering significant advantages over conventional molecular and serological approaches[46].

Treatment

Current therapeutic modalities for toxoplasmosis: Although the combination of sulfadiazine and pyrimethamine is the primary treatment regimen for toxoplasmosis, its efficiency in eliminating the infection is limited, besides causing several adverse effects, including bone marrow toxicity, pancytopenia, and megaloblastic anemia[47]. Alternative treatment regimens include pyrimethamine combined with clindamycin, clarithromycin, atovaquone, or azithromycin; however, none demonstrate efficacy against the latent tissue cyst stage of the infection[48]. Spiramycin and azithromycin are the most frequently used medicines for treating asymptomatic toxoplasmosis in the general population and in pregnant women. Nonetheless, barrier of blood-brain penetration and low bioavailability may limit the complete therapeutic efficiency of these medications[15].

Recent research indicates that spiramycin may effectively combat acute toxoplasmosis, *i.e.*, exhibiting lower toxicity, and attaining greater concentrations in the placenta compared to other medications. These traits aid in preventing parasite transfer from mother to fetus during gestation. Despite its significant benefits, penetration of the blood-brain barrier is inadequate, which necessitates its further advancement to profit from its benefits[49].

The principal adverse effect associated with extended antifolate treatment is myelotoxicity. To minimize this risk during prolonged antifolate therapy in chronic toxoplasmosis, leucovorin (folinic acid) is routinely co-administered to safeguard the host's folate pool. The incorporation of leucovorin does not interfere with the antiparasitic action of antifolates, as the parasite is believed to be incapable of utilizing reduced folate forms[50].

Ocular toxoplasmosis is traditionally managed with pyrimethamine and sulfadiazine in combination with a corticosteroid. Corticosteroids are believed to reduce intraocular inflammation. However, using corticosteroids alone was associated with worsening the disease, and leading to severe complications[51].

In fact, chemotherapy for toxoplasmosis lacks specificity, and is ineffective against some *T. gondii* forms. The treatment may hinder tachyzoite replication, but is ineffective in eradicating bradyzoites enclosed in tissue cysts. Consequently, there is a necessity for the exploration of novel therapeutic strategies for toxoplasmosis[52].

Nanotechnology applied in treatment: Successful medication delivery is facilitated by enhancing drug bioavailability and membrane permeability, and reducing drug dosages[49]. Being efficient drug delivery system, nanoparticles (NPs) showed considerable potential in overcoming the drawbacks associated with traditional anti-toxoplasmosis medications. Gained advantages include sustained release of drugs, protection from degradation, increased cellular uptake, and selective targeting of *T. gondii*-infected cells[53]. Several recent studies reported enhancement of the efficacy of treatment by using different types of NPs combined with one or the other of chemotherapeutic agents for toxoplasmosis[54-56].

The green synthesis of NPs employs natural and environmentally friendly materials, thereby reducing energy use and avoiding toxic and harmful reagents[57]. Researchers are advancing treatment options for toxoplasmosis by developing green-synthesized NPs, to enhance biomedical science and offer more effective, safer, and environmentally sustainable therapies for toxoplasmosis[58]. Metal–organic frameworks (MOFs) were also employed in various studies as nanocarriers for anti-*T. gondii* drugs. Their chemical structure can be modified by altering the metal ions and/or organic linkers, resulting in an increased surface area and, consequently, enhanced drug-loading capacity[59,60].

Drug repurposing approach: Drug repurposing are novel clinical applications for established medications with defined therapeutic purposes. This process represents a promising approach to drug re-discovery, potentially reducing financial costs and shortening development times[61]. This application experienced significant advancement over the past decade and represents a promising strategy for identifying therapeutic alternatives for rare and neglected diseases[62].

In an attempt to assess the efficacy of 666 compounds from the Selleck New Compound Library (https://www.selleckchem.com/screening/fda-approved-drug-library), 68 compounds inhibited *T. gondii* growth. Among these, NVP-AEW541 and GSK-J4 HCl specifically inhibited tachyzoite invasion and proliferation by interrupting its cell cycle progression from G1 to S phase, respectively. Both compounds extended the survival of acutely infected mice with *T. gondii*, and significantly decreased the tissue parasite burden[63]. Later, a study involving

the screening of the COVID Box (160 compounds) assessed the potential drug repurposing candidates for toxoplasmosis. The investigators proposed almitrine a promising therapeutic candidate for toxoplasmosis[64].

Altiratinib, initially developed for the treatment of glioblastoma, demonstrated significant parasiticidal activity against *T. gondii*. It was demonstrated to universally disrupt the splicing process by inhibiting a specific kinase known as *T. gondii* pre-mRNA processing factor 4 kinase. The findings indicated the necessity for continued advancement of pan-apicomplexan inhibitors aimed at this pathway[65]. Both clofazimine which serves as an anti-tuberculosis antibiotic[66], and triclabendazole which is utilized for the treatment of animal fascioliasis[67] demonstrated potential efficacy in treating toxoplasmosis. In an *in vitro* study, the investigators showed that both drugs inhibited spermine incorporation into the parasite, a process essential for the synthesis of other polyamines[62]. In another previous experimental study, the investigators evaluated the potential efficacy of clofazimine for the treatment of toxoplasmosis. Results revealed promising results, demonstrating its effectiveness in reducing cyst burden during both acute and chronic toxoplasmosis. Additionally, mice treated with clofazimine exhibited elevated levels of IFN-γ, indicating its potential immunomodulatory role[68].

Evaluation of new compounds: Tyrosine is a key amino acid required for *Toxoplasma* proliferation and the establishment of parasitophorous vacuoles. It is transformed into levodopa by two distinct aromatic amino acid hydroxylases (AAHs). Experimental assays using recombinant AAHs expressed in *E. coli*, along with two chemical derivatives (para-nitro and meta-iodo), showed that *T. gondii* AAHs can be targeted by 4-arylthiosemicarbazide derivatives[69]. A new series of (1-benzyl-4-triazolyl)-indole-2-carboxamides and structurally related compounds was investigated for their activity against toxoplasmosis. Notably, compounds JS-2-41 and JS-2-44 demonstrated significant *in vivo* effectiveness by reducing the number of *Toxoplasma* brain cysts in experimentally infected rats[70].

Natural products: Natural products served as a significant source of treatments for numerous parasitic diseases. They exhibit greater diversity, structural complexity, and molecular rigidity compared to synthetic alternatives[71]. Numerous natural products were evaluated and demonstrated promising activity in the treatment of toxoplasmosis as *Cuminum cyminum* seed oil[72], extracts of berberine[73], *Azadirachta indica*[74], as well as a combination of *Tabebuia rosea* and *Tabebuia chrysantha*[75].

Novel therapeutic targets

- Current treatments for toxoplasmosis primarily target apicoplast-based protein biosynthesis and folate metabolism. Advances in genome sequencing and molecular genetic tools have led to the identification of *T. gondii* specific proteins that play essential roles in parasite survival. These proteins represent promising targets for drug development[76].
- Acknowledgment of *T. gondii* histone deacetylases resulted in the development of a novel specific inhibitor that affects gene expression in *T. gondii* and *Plasmodium* spp. strains *in vitro* and in mouse models for acute and chronic toxoplasmosis[77].
- Protein kinases (PKs) emerged as key targets for designing highly specific and effective inhibitors against numerous diseases. Several previous studies claimed that *T. gondii* PKs were distinct from those in mammalian cells, positioning them as promising candidates for novel drug development[78]. The following are examples of these studies.

1. A study identified *T. gondii* calcium calmodulin-dependent PK (*Tg*CAMK) at the apical region of both extracellular, and intracellular tachyzoites of infected cells. The investigators proposed it a novel therapeutic target for toxoplasmosis[79].
2. *Toxoplasma* hexokinase (*Tg*HK), a PK involved in regulating glycolysis, represents promising targets for the development of new therapies. Since glycolysis plays a critical role in *T. gondii* development, its disruption impairs tachyzoite replication and tissue cyst formation, highlighting its significance as a key therapeutic target[80].
3. Calcium-dependent protein kinases (CDPKs) play essential roles in *T. gondii* intracellular development. An atypical member of this family, CDPK7, exhibits a distinct domain architecture, and composition compared to other CDPK family members. Quantitative phosphoproteomic analysis suggested that *Tg*CDPK7 had an essential role in regulating the phosphorylation of proteins potentially associated with lipid metabolism and protein/lipid transport[81].
4. Inhibition of AMP-activated PK, involved in energy metabolism impaired *Toxoplasma* growth, indicating their critical function in sustaining metabolic activity. Additionally, their distinct features in *T. gondii* may open new avenues for designing more targeted, safe, and effective treatments for toxoplasmosis[78].
5. Chinese investigators identified tRNA as a novel target within the translation machinery for potential drug development. In *T. gondii*, specific tRNAs undergo thiouracil modifications at defined sites, which are critical for their proper function. The enzymes catalyzing these modifications are essential for *Toxoplasma* survival, making them attractive targets for the development of new therapeutic agents[82].
6. Another study investigated the potential of Panobinostat (LBH589), a novel histone deacetylase (HDAC) inhibitor, for the treatment of ocular toxoplasmosis. The findings demonstrated that LBH589 suppressed *T. gondii* proliferation and activity in a dose-dependent manner, while

exhibiting minimal toxicity to retinal pigment epithelial cells[83].

- Of note, the histone code is formed through various post-translational modifications (PTMs), *e.g*., acetylation. This code is recognized by specialized proteins known as readers that regulate the structure and function of chromatin. Replacement of standard histones with variant forms introduces another level of control over gene expression. It was reported that *T. gondii* possesses a unique histone variant (H2B), termed H2B.Z. The combined influence of PTMs and histone variants is essential for gene regulation in *T. gondii*, highlighting potential targets for the development of new therapeutic strategies[84].
- Fatty acid, phospholipid, and neutral lipid metabolism are fundamental to *T. gondii* lipid metabolic processes as membrane production and crucial cellular functions such as replication, invasion, egress, cell division, and apoptosis. Disruption of these pathways can impair lipid balance and damage membrane integrity, leading to death. As such, these lipid-related mechanisms are being actively explored as targets for developing novel therapeutic drugs against toxoplasmosis[85].

Immunization

Despite significant progress in developing effective vaccines for toxoplasmosis research for new protective vaccination strategies is a challenging objective. Recent experimental approaches for the development of an effective vaccine against *T. gondii* are encouraging despite significant uncertainties owing to its complex life cycle and challenges in clinical translation[86]. Vaccine candidates such as live-attenuated vaccines[87], recombinant antigens[88], and carbohydrates[89] were utilized. Besides, various platforms were evaluated for their protective effects in animal models including DNA vaccines[90], NPs-based vaccines[91-94], and virus-like particles-based vaccines[95].

Live attenuated vaccines: Attempts at treatments with γ irradiation, chemicals, and multiple passages were employed to produce an attenuated *T. gondii* that is less virulent and unable to complete its life cycle[86]. This vaccine has limitations that include that its use is restricted to veterinary applications and it has a limited shelf life of 10 d. Additionally, being derived from a live-attenuated pathogen, the vaccine cannot be administered to humans due to safety concerns[12]. The swift advancement of gene-editing technology rendered the CRISPR/Cas9 an important transforming, potent, and precise tool for gene editing and deletion in *T. gondii*, enabling the creation of functionally live attenuated strains. The benefit of this approach is to foster development of a vaccine of low virulence while still able to stimulate host immunity[96]. A recent research by Wang *et al.*[87] tested a live attenuated vaccine of WH3 Δrop18 and their results showed that all vaccinated mice were able to survive when challenged with infection by various strains of *T. gondii*, including RH (type I), ME49 (type II), WH3 or WH6 (type Chinese 1)[87].

Recombinant antigens: A recombinant subunit vaccine demonstrated higher safety profile and unusual side effects due to its composition of highly purified antigens[88]. In fact, *T. gondii* possesses over 1000 proteins and glycoproteins that constitute a variable collection of antigens, derived from the various structures of the parasite as surface antigens, stage specific antigens and circulating antigens[97]. Protein vaccines are essentially composed of highly purified antigen. as the essential component of the vaccine. Hence, protein and subunit vaccines demonstrate a high level of safety, and a diverse range of antigens have been investigated as potential candidates for vaccination[98]. On the other hand, a recombinant cocktail protein vaccine including macrophage migration inhibitory factor (*Tg*MIF), calcium-dependent protein kinase 3 (*Tg*CDPK3), and *Tg*14-3-3 proteins was evaluated. Immunized mice with cocktail (3 proteins) vaccine elicited a strong immune response with highest levels of IgG antibody and IFN-γ production compared to controls, and other vaccines composed of two proteins[99].

Carbohydrate vaccines: It was reported that vaccines developed with glycosyl-phosphatidyl-inositol (GPI) glycoconjugates represent possible candidates, with significant advantages compared to conventional vaccine. Notably, they present a significantly higher safety profile than live attenuated or inactivated vaccines[12]. However, carbohydrates are inclined to produce lower immunogenicity compared to proteins, resulting in reduced production of high-affinity antibodies. Interestingly, the structure of carbohydrates often resembles that of the host, which may result in autoimmunity[86]. A previous study[89] also demonstrated that an immune response elicited by specific *T. gondii* GPI glycoconjugates do not confer protective immunity.

The DNA derived vaccines: They are promising platforms against toxoplasmosis being easy to produce, safe, and able to stimulate both humoral and cellular immune responses[100]. However, these vaccines generate only weak immunity due to the poor distribution of plasmids or degradation by lysosomes requiring suitable antigen delivery system to elicit optimal immune responses[101]. In a recent study[102], the investigators immunized mice with DNA vaccine encoding *T. gondii* histone deacetylase sirtuin-2 (pVAX1-SIR2) loaded on chitosan and poly (d, l-lactic-co-glycolic)-acid (PLGA) nanospheres. Results revealed that immunized mice when challenged with a lethal dose of *T. gondii* RH strain showed more resistance to infection with low parasite burden.

Nanotechnology-based vaccines: Against various pathogens, including *T. gondii*, NPs can serve as both carriers for antigen delivery, and immune-

stimulating adjuvants to boost the immune response. When administered intramuscularly, NPs exhibit a depot effect by retaining the antigen near the injection site for an extended period. This sustained presence allows the antigen to be released gradually, prolonging its availability to antigen-presenting cells leading to a stronger and more effective T-cell immune response[103].

Recently, self-assembling NPs emerged as innovative delivery systems in vaccines development. This approach facilitates efficient uptake by antigen-presenting cells, which is crucial for triggering a robust immune response[104]. Although only a few studies have investigated their application in immunization against toxoplasmosis, obtained results so far indicated potential strong and high efficacy. These studies evaluated nasal administration of porous maltodextrin NPs loaded with *T. gondii* antigen instead of traditional routes *e.g*., oral and intradermal. Potent immune response and protection against challenged infection was observed in nasal immunized animals, with recommendations for further assessment of their toxicity and safety measures[91-94].

Virus-like particle (VLP) vaccines: The use of VLP vaccines represents a novel strategy, and their continued development is recommended for vaccine development. They are highly safe because they lack the genetic material necessary for replication. Furthermore, due to their size, these particles display fast transfer to the lymph nodes leading to induction of a swift immune response. The repetitive presentation of antigens on the particle surface enhances the induction of a robust immune response[12]. In a previous study[95], a VLP vaccine expressing *T. gondii* rhoptry-13 (ROP13) were generated. Mice immunized elicited significantly higher levels of *T. gondii*-specific antibodies following boost immunization, whereas no significant antibody inductions were detected upon prime immunization.

CONCLUDING REMARKS

1. Heat therapies, radiation and pressure treatments may serve as effective alternatives against *T. gondii* oocysts in water and vegetables. However, extreme heat or cold can cause *T. gondii* encysted in meat to perish. Therefore, meat should be cooked at 67°C or stored at -13°C before consumption.
2. Screening for *T. gondii* antibodies in blood as well as blood products should be incorporated into the pre-transfusion blood testing protocol and leukoreduction filters could reduce the risk of toxoplasmosis.
3. Prevention of toxoplasmosis in solid organ transplant patients requires multiple strategies, including serologic screening of both donors and recipients, chemoprophylaxis, and ongoing serological monitoring of patient's post-transplantation.
4. Recent technology, *e.g.*, INAAT utilizing either LAMP and NASBA is a promising tool for diagnosing toxoplasmosis. In addition, miRNAs proved potential biochemical markers for toxoplasmosis.
5. Since NPs serve as efficient delivery systems, nanotechnology provided wide diversity of applications in all eras of control measures (diagnosis, treatment and prevention) against toxoplasmosis.
6. Several approved drugs were investigated for their potential efficacy against toxoplasmosis such as NVP-AEW541, GSK-J4 HCl, almitrine, altiratinib, clofazimine, and triclabendazole.
7. Aromatic amino acid hydroxylases and series of (1-benzyl-4-triazolyl)-indole-2-carboxamides and structurally related compounds were investigated for their activity that showed potential efficacy against toxoplasmosis.
8. Numerous natural products demonstrated promising activity in the treatment of toxoplasmosis as *C. cyminum* seed oil, and extracts of berberine, *A. indica, T. rosea* and *T. chrysantha*. Moreover, they exhibit greater diversity, structural complexity, and molecular rigidity compared to synthetic alternatives.
9. Advances in genome sequencing and molecular genetic tools resulted in identification of *T. gondii* specific drug targets, such as PKs, histone modifying enzymes, tRNA target, and lipid-related mechanisms.
10. Various vaccine platforms were experimentally evaluated for production of effective vaccines for toxoplasmosis, such as NPs-based vaccines, DNA vaccines, and virus-like particles-based vaccines.

Author's contribution: All authors contributed to retrieving data, composing the review, and approved the final version of the manuscript before publication.
Competing interests: None.
Funding statement: None.

REFERENCES

1. Wang JL, Liang QL, Li TT, He JJ, Bai MJ, Cao XZ, *et al. Toxoplasma gondii* tkl1 deletion mutant is a promising vaccine against acute, chronic, and congenital toxoplasmosis in mice. J Immunol 2020; 204(7):1562-1570.
2. Dubois D, Soldati D. Biogenesis and secretion of micronemes in *Toxoplasma gondii*. Cell Microbiol 2019; 21(5):e13018.
3. Hatam-Nahavandi K, Calero-Bernal R, Rahimi MT, Pagheh AS, Zarean M, Dezhkam A, *et al. Toxoplasma gondii* infection in domestic and wild felids as public health concerns: A systematic review and meta-analysis. Sci Rep 2021; 11(1):9509.
4. Lazar LT, Al-Ammash MS, Abas KS. *Toxoplasma gondii*: Life cycle, pathogenesis, and immune response: A review. Plant Arch 2021; 21(1):1-10.
5. Mose JM, Kagira JM, Kamau DM, Maina NW, Ngotho M, Karanja SM. A review on the present advances on studies of toxoplasmosis in Eastern Africa. Biomed Res Int 2020; 2020:7135268.

6. Kuruca L, Belluco S, Vieira-Pinto M, Antic D, Blagojevic B. Current control options and a way towards risk-based control of *Toxoplasma gondii* in the meat chain. Food Control 2023; 146:109556.
7. Shapiro K, Bahia-Oliveira L, Dixon B, Dumètre A, de Wit LA, VanWormer E, *et al.* Environmental transmission of *Toxoplasma gondii*: Oocysts in water, soil and food. Food Waterborne Parasitol 2019; 15:e00049.
8. Pinto-Ferreira F, Paschoal ATP, Pasquali AKS, Bernardes JC, Caldart ET, Freire RL, *et al.* Techniques for inactivating *Toxoplasma gondii* oocysts: A systematic review. Braz J Vet Parasitol 2021; 30(2):e026420.
9. Barros M, Teixeira D, Vilanova M, Correia A, Teixeira N, Margarida B. Vaccines in congenital toxoplasmosis: Advances and perspectives. Front Immunol 2020; 11:621.
10. Layton J, Theiopoulou DC, Rutenberg D, Elshereye A, Zhang Y, Sinnott J, *et al.* Clinical spectrum, radiological findings, and outcomes of severe toxoplasmosis in immunocompetent hosts: A systematic review. Pathogens 2023; 12(4):543.
11. Bollani L, Auriti C, Achille C, Garofoli F, De Rose DU, Meroni V, *et al.* Congenital toxoplasmosis: The state of the art. Front Pediatr 2022; 10:894573.
12. Chu KB, Quan FS. Advances in *Toxoplasma gondii* vaccines: Current strategies and challenges for vaccine development. Vaccines 2021; 9(5):413.
13. Innes EA, Hamilton C, Garcia JL, Chryssafidis A, Smith D. A one health approach to vaccines against *Toxoplasma gondii*. Food Waterborne Parasitol 2019; 15:e00053.
14. Dunay IR, Gajurel K, Dhakal R, Liesenfeld O, Montoya JG. Treatment of toxoplasmosis: Historical perspective, animal models, and current clinical practice. Clin Microbiol Rev 2018; 31(4):e00057-17.
15. Allam AF, Shehab AY, Mogahed NM, Farag HF, Elsayed Y, Abd El-Latif NF. Effect of nitazoxanide and spiramycin metronidazole combination in acute experimental toxoplasmosis. Heliyon 2020; 6(4):e03661.
16. Safarpour H, Cevik M, Zarean M, Barac A, Hatam-Nahavandi K, Rahimi MT, *et al.* Global status of *Toxoplasma gondii* infection and associated risk factors in people living with HIV. AIDS 2020; 34(3):469-474.
17. Taman A, Alhusseiny SM. Exposure to toxoplasmosis among the Egyptian population: A systematic review. Parasitol United J 2020; 13(1):1-10.
18. Blackburn D, Mba N, Nwachukwu W, Zhou H, Hill A, Abbott A, *et al.* Seroprevalence and risk factors for *Toxoplasma gondii* infection in women of reproductive age in Nigeria in 2018. Am J Trop Med Hyg 2024; 111(5):1005-1014.
19. Bahadori A, Babazadeh T, Chollou KM, Moqadam H, Zendeh MB, Valipour B, *et al.* Seroprevalence and risk factors associated with toxoplasmosis in nomadic, rural, and urban communities of northwestern Iran. Front Public Health 2025; 13:1516693.
20. Nakajo K, Nishiura H. *Toxoplasma gondii* infection risk among pregnant people and congenital toxoplasmosis incidence in Japan. Epidemiol Infect 2025; 153:e74.
21. Manuel L, Gomes SG, Mahomed NE. Human toxoplasmosis in Mozambique: Gaps in knowledge and research opportunities. Parasit Vectors 2020; 13:571.
22. Sawers L, Wallon M, Mandelbrot L, Villena I, Stillwaggon E, Kieffer F. Prevention of congenital toxoplasmosis in France using prenatal screening: A decision-analytic economic model. PLoS ONE 2022; 17(11):e0273781.
23. Etewa SE, Sarhan MH, Moawad HS, Mohammad SM, Samira MA, Samir MA, *et al.* Behavior and neuropsychiatric changes in experimental chronic toxoplasmosis: Histopathological and immunohistochemical studies. Parasitol United J 2021; 14(2):183-192.
24. Grada S, Mihu AG, Petrescu C, Suciu O, Marincu I, Lupu MA, *et al. Toxoplasma gondii* infection in patients with psychiatric disorders from Western Romania. Medicina 2022; 58:208.
25. Esboei RB, Fallahi S, Zarei M, Kazemi B, Mohebali M, Shojaee S, *et al.* Utility of blood as the clinical specimen for the diagnosis of ocular toxoplasmosis using uracil DNA glycosylase-supplemented loop-mediated isothermal amplification and real-time polymerase chain reaction assays based on REP-529 sequence and B1 gene. BMC Infect Dis 2022; 22:89.
26. Kalogeropoulos D, Sakkas H, Mohammed B, Vartholomatos G, Malamos K, Sreekantam S, *et al.* Ocular toxoplasmosis: A review of the current diagnostic and therapeutic approaches. Int Ophthalmol 2022; 42:295-321.
27. Vanessa SM. The important role of laboratories in the diagnosis and prevention of *Toxoplasma* infection: Towards new perspectives on *Toxoplasma gondii*. Intechopen 2022; Available online at: http://dx.doi.org/10.5772/intechopen.108313.
28. Rostkowska C, Mota CM, Oliveira TC, Santiago FM, Oliveira LA, Korndörfer GH, *et al.* Si-accumulation in *Artemisia annua* glandular trichomes increases artemisinin concentration, but does not interfere in the impairment of *Toxoplasma gondii* growth. Front Plant Sci 2016; 7:1430.
29. ElBahaie ES, Ahmed FA, Ibrahim AI, Abdel-Rahman EM. Toxoplasmosis and iron chelation in *Toxoplasma* infection: Overview. J Pharm Negat 2023; 14(3): 727-732.
30. Guegan H, Robert-Gangneux F. *Toxoplasma gondii* in solid organ and stem cell transplant: Prevention and treatment. In Morris MI, Kotton CN, Wolfe C (Eds.), Emerging Transplant Infections, Springer International Publishing. Available online at: https://doi.org/10.1007/978-3-030-01751-4_51-1.
31. Ramanan P, Scherger S, Benamu E, Bajrovic V, Jackson W, Hage CA, *et al.* Toxoplasmosis in non-cardiac solid organ transplant recipients: A case series and review of literature. Transpl Infect Dis 2020; 22(1):e13218.
32. Belkacemi M, Heddi B. Toxoplasmosis immunity status of blood donors in Sidi Bel Abbès, West Algeria. Cureus 2022; 14(9):e28826.
33. Mardani A. Prevention strategies of transfusion-transmitted parasitic infections (TTPIs): Strengths and challenges of current approaches, and evaluation of the strategies implemented in Iran. Parasite Epidemiol Control 2020; 9:e00141.
34. Qi T, Ai J, Sun Y, Ma H, Kang M, You X, Li J. Application of *Toxoplasma gondii*-specific SAG1, GRA7 and BAG1

proteins in serodiagnosis of animal toxoplasmosis. Front Cell Infect Microbiol 2022; 12:1029768.
35. Wesołowski R, Pawłowska M, Smoguła M, Szewczyk-Golec K. Advances and challenges in diagnostics of toxoplasmosis in HIV-infected patients. Pathogens 2023; 12(1):110.
36. Mihu AG, Lupu MA, Nesiu A, Marti DT, Olariu TR. Screening for the detection of *Toxoplasma gondii* IgG, IgM and IgA in females of reproductive age from Western Romania. Life 2022; 12(11):1771.
37. Ismael SS. Diagnostic methods and protocols used in investigating *Toxoplasma gondii* in humans: A review. Baghdad J Biochem Appl Biol Sci 2021; 2(4):181-186.
38. Hegazy MK, Awad SI, Saleh NE, Hegazy MM. Loop Mediated Isothermal Amplification (LAMP) of *Toxoplasma* DNA from Dried Blood Spots. Exp Parasitol 2020; 211:107869.
39. Norouzi R. Comparison of a nucleic acid sequence-based amplification (NASBA) and real-time reverse transcriptase PCR methods for detection of *Toxoplasma gondii* in rat blood samples. J Zoonotic Dis 2016; 1(1): 15-23.
40. Mogahed NM, Khedr SI, Ghazala R, Masoud IM. Can miRNA712_3p be a promising biomarker for early diagnosis of toxoplasmosis? Asian Pac J Trop Med 2018; 11(12):688-692.
41. Mady RF, El-Temsahy MM, Issa YA, Zaghloul AS, Khedr SI. MicroRNA mmu-miR-511-5p: A promising diagnostic biomarker in experimental toxoplasmosis using different strains and infective doses in mice with different immune states before and after treatment. Acta Parasitol 2024; 69:1253-1266.
42. Galal L, Ariey F, Gouilh MA, Dardé ML, Hamidović A, Letourneur F, *et al.* A unique *Toxoplasma gondii* haplotype accompanied the global expansion of cats. Nat Commun 2022; 13(1):5778.
43. Ekawasti F, Cahyaningsih U, Dharmayanti NLPI, Sa'diah S, Subekti DT, Azmi Z, *et al.* Restriction fragment length polymorphism analysis of genes of virulent strain isolate of *Toxoplasma gondii* using enzyme DdeI. Int J One Health 2021; 7(2):196-203.
44. Assolini JP, Concato VM, Gonçalves MD, Carloto ACM, Conchon-Costa I, Pavanelli WR, *et al.* Nanomedicine advances in toxoplasmosis: diagnostic, treatment, and vaccine applications. Parasitol Res 2017; 116:1603-1615.
45. Jazayeri MH, Aghaie T, Avan A, Vatankhah A, Ghaffari MRS. Colorimetric detection based on gold nanoparticles (GNPs): An easy, fast, inexpensive, low-cost and short time method in detection of analytes (protein, DNA, and ion). Sens Bio-Sens Res 2018; 20:1-8.
46. Sousa S, Castro A, Correia da Costa JM, Pereira E. Biosensor based immunoassay: A new approach for serotyping of *Toxoplasma gondii*. Nanomaterials 2021; 11(8):2065.
47. Saad AE, Zoghroban HS, Ghanem HB, El-Guindy DM, Younis SS. The effects of L-citrulline adjunctive treatment of *Toxoplasma gondii* RH strain infection in a mouse model. Acta Trop 2023; 239:106830.
48. El-Kady AM, Al-Megrin WA, Abdel-Rahman IA, Sayed E, Alshehri EA, Wakid MH, *et al.* Ginger is a potential therapeutic for chronic toxoplasmosis. Pathogens 2022; 11(7):798.
49. Hagras NA, Mogahed NM, Sheta E, Darwish AA, El-hawary MA, Hamed MT, *et al.* The powerful synergistic effect of Spiramycin/propolis loaded chitosan/alginate nanoparticles on acute murine toxoplasmosis. PLoS Negl Trop Dis 2022; 16(3):e0010268.
50. Dian S, Ganiem AR, Ekawardhani S. Cerebral toxoplasmosis in HIV-infected patients: A review. Pathog Glob Health 2023; 117(1):14-23.
51. Lin HY, Lee WA. The role of corticosteroids in treating acute ocular toxoplasmosis in an immunocompetent patient: A case report. Front Med 2022; 9:843050.
52. El Sharazly BM, Aboul Asaad IA, Yassen NA, El Maghraby GM, Carter WG, Mohamed DA, *et al.* Mefloquine loaded niosomes as a promising approach for the treatment of acute and chronic toxoplasmosis. Acta Trop 2023; 239:106810.
53. Ismael SS. Role of nanotechnology in treating of *Toxoplasma gondii*. In: Abbas RZ, Hassan MF, Khan A, Mohsin M (Eds.), Zoonosis, 1st edition; Unique Scientific Publishers, Faisalabad, Pakistan 2023.
54. Etewa SE, El-Maaty DAA, Hamza RS, Metwaly AS, Sarhan MH, Abdel-Rahman SA, *et al.* Assessment of Spiramycin-loaded chitosan nanoparticles treatment on acute and chronic toxoplasmosis in mice. J Parasit Dis 2018; 42(1):102-113.
55. Abdel-Wahab A, Shafey D, Selim S, Sharaf S, Mohsen K, Allam D, *et al.* Spiramycin-loaded maltodextrin nanoparticles as a promising treatment of toxoplasmosis on murine model. Parasitol Res 2024; 123:286.
56. Osama D, El-Dib N, khattab H, Ibrahim H, khater M, Elmahallawy E, *et al.* Spiramycin-chitosan nanoparticles decline parasite burden and renovate patent histopathological changes in liver and lung in mice experimentally infected with acute toxoplasmosis. EJVS 2024; 55(4):1151-1164.
57. Ying S, Guan Z, Ofoegbu PC, Clubb P, Rico C, He F, *et al.* Green synthesis of nanoparticles: Current developments and limitations. Environ Technol Innov 2022; 26:102336.
58. Alshamrani M. Targeted drug delivery with green nanoparticles: A new frontier in *Toxoplasma gondii* infection treatment. Pak Vet J 2025; 45(1):84-95.
59. El-Shafey AAM, Hegab MHA, Seliem MME, Barakat AMA, Mostafa NE, Abdel-Maksoud HA, *et al.* Curcumin@metal organic frameworks nano-composite for treatment of chronic toxoplasmosis. J Mater Sci Mater Med 2020 31(11):90.
60. El Naggar HM, Anwar MM, Khayyal AE, Abdelhameed RM, Barakat AM, Sadek SAS, *et al.* Application of honeybee venom loaded nanoparticles for the treatment of chronic toxoplasmosis: Parasitological, histopathological, and immunohistochemical studies. J Parasit Dis 2023; 47(3):591-607.
61. Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, *et al.* Drug repurposing: Progress, challenges

and recommendations. Nat Rev Drug Discov 2019; 18(1):41-58.
62. Corvi MM, Rossi F, Ganuza A, Alonso AM, Alberca LN, Dietrich RC, *et al.* Triclabendazole and clofazimine reduce replication and spermine uptake *in vitro* in *Toxoplasma gondii*. Parasitol Res 2024; 123:69.
63. Liu S, Wu M, Hua Q, Lu D, Tian Y, Yu H, *et al.* Two old drugs, NVP-AEW541 and GSK-J4, repurposed against the *Toxoplasma gondii* RH strain. Parasit Vectors 2020; 13(1):242.
64. Dos Santos BR, Ramos ABSB, de Menezes RPB, Scotti MT, Colombo FA, Marques MJ, *et al.* Repurposing the medicines for malaria venture's COVID box to discover potent inhibitors of *Toxoplasma gondii*, and *in vivo* efficacy evaluation of almitrine bismesylate (MMV1804175) in chronically infected mice. PLoS One 2023; 18(7):e0288335.
65. Swale C, Bellini V, Bowler MW, Flore N, Brenier-Pinchart MP, Cannella D, *et al.* Altiratinib blocks *Toxoplasma gondii* and *Plasmodium falciparum* development by selectively targeting a spliceosome kinase. Sci Transl Med 2022; 14(656):eabn3231.
66. Mirnejad R, Asadi A, Khoshnood S, Mirzaei H, Heidary M, Fattorini L, *et al.* Clofazimine: A useful antibiotic for drug-resistant tuberculosis. Biomed Pharmacother 2018; 105:1353-1359.
67. Rodríguez-Hidalgo R, Calvopiña M, Romero-Alvarez D, Montenegro-Franco M, Pavon D, Pointier JP, *et al.* Triclabendazole efficacy, prevalence, and re-infection of *Fasciola hepatica* in bovine and ovine naturally infected in the Andes of Ecuador. Vet Parasitol Reg Stud Rep 2024; 47:100947.
68. Biglari-Moghadam N, Najafzadehvarzi H, Gorgani-Firouzjaee T, Ghasemi-Kasman M. Efficacy of Clofazimine against acute and chronic *Toxoplasma gondii* infection in mice. Microb Pathog 2023; 181:106206.
69. Bekier A, Brzostek A, Paneth A, Dziadek B, Dziadek J, Gatkowska J, *et al.* 4-Arylthiosemicarbazide derivatives as toxoplasmic aromatic amino acid hydroxylase inhibitors and anti-inflammatory agents. Int J Mol Sci 2022; 23(6):3213.
70. Khan SM, Hernandez GA, Allaie IM, Grooms GM, Li K, Witola WH, Stec J. Activity of (1-benzyl-4-triazolyl)-indole-2-carboxamides against *Toxoplasma gondii* and *Cryptosporidium parvum*. Int J Parasitol Drugs Drug Resist 2022; 19:6-20.
71. Atanasov AG, Zotchev SB, Dirsch VM, Supuran CT. Natural products in drug discovery: Advances and opportunities. Nat Rev Drug Discov 2021; 20(3):200-216.
72. Gomaa MM, Sheta E. Efficacy of *Cuminum cyminum* (L.) seed oil on acute toxoplasmosis: An experimental study on albino mice. Parasitol United J 2022; 15(1):89-109
73. Abd El Wahab WM, Ismail MAM, Zalat RS, Ghieth MA, Abdel Gawad SS, Ahmed MM, *et al.* Prophylactic and therapeutic efficacy of berberine on chronic toxoplasmosis in diabetic or hypertensive mice. Parasitol United J 2024; 17(2):126-134.
74. Rashed HA, Younis SS, Abdel Bary A, Elmorsy AE. *Azadirachta indica* (Neem) extract as natural agent against experimental toxoplasmosis. Parasitol United J 2023; 16(3):253-260.
75. Cardona-Trujillo MC, Jiménez-González FJ, Veloza LA, Sepúlveda-Arias JC. *In vitro* anti-*Toxoplasma* activity of extracts obtained from *Tabebuia rosea* and *Tabebuia chrysantha*: The role of β-amyrin. Molecules 2024; 29(5):920.
76. Müller J, Hemphill A. *Toxoplasma gondii* infection: Novel emerging therapeutic targets. Expert Opin Ther Targets 2023; 27(4-5):293-304.
77. Jublot D, Cavaillès P, Kamche S, Francisco D, Fontinha D, Prudêncio M, *et al.* A histone deacetylase (HDAC) inhibitor with pleiotropic *in vitro* anti-*Toxoplasma* and anti-*Plasmodium* activities controls acute and chronic *Toxoplasma* infection in mice. Int J Mol Sci 2022; 23(6):3254.
78. Dos Santos DA, Souza HFS, Silber AM, de Souza TACB, Ávila AR. Protein kinases on carbon metabolism: Potential targets for alternative chemotherapies against toxoplasmosis. Front Cell Infect Microbiol 2023; 13:1175409.
79. Kato K, Sugi T, Takemae H, Takano R, Gong H, Ishiwa A, *et al.* Characterization of a *Toxoplasma gondii* calcium calmodulin-dependent protein kinase homolog. Parasit Vectors 2016; 9:405.
80. Shukla A, Olszewski K L, Llinás M, Rommereim L M, Fox B A, Bzik D J, *et al.* Glycolysis is important for optimal asexual growth and formation of mature tissue cysts by *Toxoplasma gondii*. Int J Parasitol 2018; 48:955–968.
81. Bansal P, Antil N, Kumar M, Yamaryo-Botté Y, Rawat RS, Pinto S, *et al.* Protein kinase *Tg*CDPK7 regulates vesicular trafficking and phospholipid synthesis in *Toxoplasma gondii*. PLoS Pathog 2021; 17(2):e1009325.
82. Yang Y, Lin M, Chen X, Zhao X, Chen L, Zhao M, *et al.* The first apicoplast tRNA thiouridylase plays a vital role in the growth of *Toxoplasma gondii*. Front Cell Infect Microbiol 2022; 12:947039.
83. Zhang Y, Zhang Q, Li H, Cong H, Qu Y. *In vitro* and *in vivo* anti-*Toxoplasma* activities of HDAC inhibitor Panobinostat on experimental acute ocular toxoplasmosis. Front Cell Infect Microbiol 2022; 12:1002817.
84. Vanagas L, Muñoz D, Cristaldi C, Ganuza A, Nájera R, Bonardi MC, *et al.* Histone variant H2B.Z acetylation is necessary for maintenance of *Toxoplasma gondii* biological fitness. Biochim Biophys Acta Gene Regul Mech 2023; 1866(3):194943.
85. He TY, Li YT, Liu ZD, Cheng H, Bao YF, Zhang JL. Lipid metabolism: The potential targets for toxoplasmosis treatment. Parasit Vectors 2024; 17:111.
86. Zhang Y, Li D, Lu S, Zheng B. Toxoplasmosis vaccines: What we have and where to go? NPJ Vaccines 2022; 7:131.
87. Wang C, Fu S, Yu X, Zhou H, Zhang F, Song L, *et al.* *Toxoplasma* WH3 Δrop18 acts as a live attenuated vaccine against acute and chronic toxoplasmosis. NPJ Vaccines 2024; 9:197.
88. Tian Y, Hu D, Li Y, Yang L. Development of therapeutic vaccines for the treatment of diseases. Mol Biomed 2022; 3(1):40.

89. Götze S, Reinhardt A, Geissner A, Azzouz N, Tsai YH, Kurucz R, Varón Silva D, Seeberger PH. Investigation of the protective properties of glycosylphosphatidylinositol-based vaccine candidates in a *Toxoplasma gondii* mouse challenge model. Glycobiology. 2015 Sep;25(9):984-91.
90. Hasan T, Kawanishi R, Akita H, Nishikawa Y. *Toxoplasma gondii* GRA15 DNA vaccine with a liposomal nanocarrier composed of an SS-cleavable and pH-activated lipid-like material induces protective immunity against toxoplasmosis in mice. Vaccines (Basel) 2021;10(1):21.
91. Ducournau C, Nguyen TT, Carpentier R, Lantier I, Germon S, Précausta F, *et al.* Synthetic parasites: A successful mucosal nanoparticle vaccine against *Toxoplasma* congenital infection in mice. Future Microbiol 2017; 12(5):393-405.
92. Ducournau C, Moiré N, Carpentier R, Cantin P, Herkt C, Lantier I, *et al.* Effective nanoparticle-based nasal vaccine against latent and congenital toxoplasmosis in sheep. Front Immunol 2020; 11:2183.
93. Ducournau C, Cantin P, Alerte V, Quintard B, Popelin-Wedlarski F, Wedlarski R, *et al.* Vaccination of squirrel monkeys (*Saimiri* spp.) with nanoparticle-based *Toxoplasma gondii* antigens: New hope for captive susceptible species. Int J Parasitol 2023; 53:333-346.
94. Abdel-Wahab A, Shafey D, Selim S, Sharaf S, Mohsen K, Allam D, *et al.* Intranasal versus intradermal immuno-protective effect of maltodextrin nanoparticles loaded with SAG1 against toxoplasmosis on murine model. Parasitol United J 2024; 17(3):189-195.
95. Kang HJ, Chu KB, Lee SH, Kim MJ, Park H, Jin H, *et al.* Virus-like particle vaccine containing *Toxoplasma gondii* rhoptry protein 13 induces protection against *T. gondii* ME49 infection in mice. Korean J Parasitol 2019; 57(5):543-547.
96. Liu J, Li TT, Liang QL, Elsheikha HM, Zhao DY, Zhang ZW, *et al.* Characterization of functions in parasite growth and virulence of four *Toxoplasma gondii* genes involved in lipid synthesis by CRISPR-Cas9 system. Parasitol Res 2021; 120:3749-3759.
97. Elmowafy AR, Sadek AG, Samn AA, Hamza H, Ibrahim RA. Assessment of the effectiveness of *Toxoplasma* surface antigen grade I for diagnosis of human *Toxoplasma gondii*. AIM J 2020; 15(1):293-298.
98. Vartak A, Sucheck SJ. Recent advances in subunit vaccine carriers. Vaccines 2016; 4(2):12.
99. Liu F, Wu M, Wang J, Wen H, An R, Cai H, *et al.* Protective effect against toxoplasmosis in BALB/c mice vaccinated with recombinant *Toxoplasma gondii* MIF, CDPK3, and 14-3-3 protein cocktail vaccine. Front Immunol 2021; 12:755792.
100. Gül C, Karakavuk T, Karakavuk M, Can H, Değirmenci Döşkaya A, Gül A, *et al.* An overview of DNA vaccines development studies against *Toxoplasma gondii*. Turkiye Parazitol Derg 2022; 46(3):253-270.
101. Sun H-C, Huang J, Fu Y, Hao L-L, Liu X, Shi T-Y. Enhancing immune responses to a DNA vaccine encoding *Toxoplasma gondii* GRA7 using calcium phosphate nanoparticles as an adjuvant. Front Cell Infect Microbiol 2021;11:787635.
102. Yu Z, Lu Y, Cao W, Aleem MT, Liu J, Luo J, *et al.* Nano DNA vaccine encoding *Toxoplasma gondii* histone deacetylase SIR2 enhanced protective immunity in mice. Pharmaceutics 2021; 13(10):1582.
103. Brito C, Lourenço C, Magalhães J, Reis S, Borges M. Nanoparticles as a delivery system of antigens for the development of an effective vaccine against *Toxoplasma gondii*. Vaccines 2023; 11(4):733.
104. Lung P, Yang J, Li Q. Nanoparticle formulated vaccines: Opportunities and challenges. Nanoscale 2020; 12(10):5746-5763.