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Current vaccine candidate of toxoplasmosis

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ABSTRACT: ***Toxoplasma gondii*** **is an intracellular protozoan parasite belonging from the phylum Apicomplexa, known for causing toxoplasmosis. The disease has a global presence, affecting about one-third of the world's population. The parasite infects various intermediate hosts, including humans and other warm-blooded mammals, with cats serving as the definitive hosts. Its life cycle is complex, featuring a sexual phase in the definitive host and an asexual phase in intermediate hosts. Toxoplasmosis can leads severe neurologic, ocular, and systemic diseases in neonates and immunocompromised individuals. In immunocompetent individuals, the infection is typically asymptomatic, forming dormant tissue cysts in immune-privileged sites such as the muscles and brain. During pregnancy, toxoplasmosis poses significant health risks, potentially causing severe birth defects or miscarriage, and a major concern for immunocompromised hosts. Current control measures are inadequate, highlighting the need for effective vaccines. The initial host defense against *T. gondii* occurs at the intestinal mucosa, where cytokines and chemokines released by intestinal epithelial cells facilitate the migration of inflammatory cells, including macrophages, neutrophils, and dendritic cells. Developing a vaccine that can enhance this mucosal immunity is crucial for preventing toxoplasmosis. Therefore, the development of vaccines against *T.gondii* is a promising alternative mechanism to prevent toxoplasmosis. This review aims to present the current status of vaccine candidates against *Toxoplasma gondii*.**

KEYWORDS: Vaccine, Candidate, Toxoplasmosis, *Toxoplasma gondii*

INTRODUCTION

Toxoplasma gondii is an intracellular protozoan parasite from phylum Apicomplexa. It causes toxoplasmosis, a parasitic disease with a global reach, impacting one-third of the world's population. It has a variety of intermediate hosts, including warm-blooded mammals and humans, with cats being the definitive hosts. The parasite has a complex life cycle, undertaking a sexual phase in the feline definitive host and an asexual phase in intermediate hosts [1].

The prevalence of *T. gondii* infection varies significantly across different countries and even among communities in the same region. In regions such as Southeast Asia, Northern Europe, and, North America a low sero-prevalence of 10–30% has been reported. Moderate prevalence (30-50%) is found in countries in southern and Central Europe, while high prevalence is seen in tropical African and Latin American countries [2].

Toxoplasmosis can cause severe ocular, neurologic and systemic diseases particularly in individuals with weakened immune systems and neonates. In immunocompetent individuals, *T. gondii* infection has two clinical stages: acute toxoplasmosis and latent toxoplasmosis [3].

Acute toxoplasmosis is often asymptomatic in healthy adults; however, symptoms may manifest as a mild, flu-like illness with low-grade fever, myalgia, malaise, and headache [4]. Latent toxoplasmosis has mild, flu-like symptoms. In the latent phase, bradyzoites cause lesions in skeletal muscle, the heart and the central nervous system, including the brain [5]. In humans, primary infection is usually subclinical. However some patients may present with ocular or cervical lymphadenopathy. Infections acquired during pregnancy can cause severe damage

to the fetus. In immunocompromised patients, reactivation of a latent infection can cause life-threatening encephalitis [6].

Toxoplasma gondii has three infective stages: tachyzoite, bradyzoites, and oocyst. All stages are infectious for both definitive and intermediate hosts. *Toxoplasma gondii* infection is mainly acquired through one of the following routes: oral ingestion of infectious oocyst from the environment, oral ingestion of tissue cysts contained in raw or undercooked meat, and transplacental transmission of tachyzoite. Additionally, tachyzoite may also be transmitted in the milk from mother to child [7]. The life cycle of *T. gondii* is facultative heteroxenous, with intermediate hosts being all warm-blooded animals, including humans, and definitive hosts are members of the family Felidae [1, 7]. *Toxoplasma gondii* undergoes two stages of asexual development in the intermediate host. In the first stage, tachyzoite multiplies rapidly within various types of host cells. Tachyzoite of the last generation initiates the second stage of development, resulting in the formation of tissue cysts. Within the tissue cyst, bradyzoites multiply slowly [8]. Bradyzoites have a high affinity for neural and muscular tissues and are predominantly located in the central nervous system (CNS), the eye, as well as skeletal and cardiac muscles. To a lesser extent, they can also be found in visceral organs such as the liver, lungs, and kidneys [9]. Bradyzoites are the terminal life cycle stage in the intermediate host and are immediately infectious. In some intermediate host species, they persist for the life of the host. Tissue cysts periodically break down, with bradyzoites transforming into tachyzoite that reinvade host cells and again transform into bradyzoites, forming new tissue cysts [10]. If ingested by a definitive host, the bradyzoites initiate another sexual stage of multiplication in the epithelial cells of the small intestine. Oocyst are released into the intestinal lumen and passed with the feces into the environment. Sporogony occurs outside the host, leading to the development of infectious oocyst, which contain two sporocysts, each with four sporozoites [11]. Oocysts are shed in the cat's feces, with large numbers being shed for 1-2 weeks. Oocyst takes 1–5 days to sporulate in the environment and become infective. Intermediate hosts become infected after ingesting water, plant material, or soil contaminated with oocyst. Oocyst converts into tachyzoite after ingestion. These tachyzoites localize in muscle and neural tissue and develop into bradyzoites. Cats become infected after consuming intermediate hosts containing tissue cysts. Cats may also become infected directly by ingesting sporulated oocyst. Tachyzoite in pregnant women are capable of infecting the fetus. *Toxoplasma gondii* may also be transmitted during a blood or organ transplant [12].

The diagnosis of *T. gondii* infection is mainly established by direct evidence showing the presence of the parasite using immunoperoxidase staining or its DNA by PCR in body fluids or tissues, or by indirect confirmation showing the presence of antibodies against the parasite [13].

The most effective treatment for toxoplasmosis involves a combination of the oral antibiotics pyrimethamine and sulfadiazine. While these anti-parasitic drugs are effects do not eradicate *T. gondii* cysts in infected hosts and can cause side effects such as toxicity and hypersensitivity [14]. Consequently, development of vaccines against *T. gondii* represents a promising alternative strategy for preventing toxoplasmosis. This review highlights the current status of vaccine candidates against toxoplasmosis, with an emphasis on the most common *T. gondii* antigens.

MECHANISMS OF PROTECTIVE IMMUNITY AGAINST TOXOPLASMOSIS

Toxoplasma gondii infection rarely produces clinical signs in the host, and the severity of the disease depends on the age of the host, species, pregnancy, nutritional and immunological status of the host, concomitant infection, and parasite stage. The mechanisms involved in host protection against infection encompass elements of the cell-autonomous , acquired and innate immune responses [15].

Innate immunity: The initial line of host defense against *T. gondii* occurs at the mucosa of the intestinal mucosa. Cytokines and chemokines secreted by intestinal epithelial cells facilitate the migration of inflammatory cells, including dendritic cells, macrophages, and neutrophils. These innate immune cells produce IL-12 (interleukin-12), which stimulates natural killer cells (NKs) and T cells to produce IFN-γ (interferon gamma). This pro-inflammatory cytokine IFN-γ is a major mediator of resistance to *T. gondii* [16].

Acquired Immunity: The activation of specific immune cells, including T and B cells. Antigen-presenting cells, such as macrophages and dendritic cells activate T cells and promote the development of Th1 cells and antigen-specific CD8+ T cells. These T cells produce interferon-gamma (IFN-γ), which is crucial for controlling *T. gondii* infection. Effective host protection against *T. gondii* relies on a robust Th1-type immune response. In this response, CD8+ T cells play a pivotal role by secreting IFN-γ and exerting direct cytotoxic effects on infected cells [17].

Cell-Autonomous Immunity: This is a critical aspect of the host's defense against *T. gondii*. This process involves the direct action of infected cells to control and eliminate the parasite. Key mediators of this immunity include free radicals, nitric oxide (NO), indoleamine 2, 3-dioxygenase (IDO), and interferon-gamma-inducible GTPases (IRGs/GBPs). These molecules help suppress *T. gondii* growth and facilitate its destruction within infected cells.

Toxoplasma gondii triggers a robust cell-mediated immune response. Tachyzoite stimulates macrophages to produce IL-12 and TNF-α, which subsequently activate NK cells and T cells to produce IFN-γ. This cytokine is essential for resistance, working in synergy with TNF-α to enhance the production of free radicals and nitric oxide (NO) by macrophages, thereby effectively killing the parasite. CD8+ T cells are the primary effector cells in this response, with CD4+ T cells playing a supportive role[18].During the early stages of infection, innate immune mechanisms are activated, primarily involving dendritic cells and macrophages [19, 20]. Endosomal toll-like receptor 11 engages with tachyzoite products, leading to the production of IL-12 and TNF-α, which then activate NK cells to secrete IFN-γ, stimulating T-cell activation[21].As the infection progresses, acquired immunity develops, characterized by strong CD4+ and CD8+ T cell activity[22]. IFN-γ remains central to resistance, promoting the differentiation of CD4+ T lymphocytes into a Th1 cytokine profile. Newly generated CD8+ T cells are crucial for controlling parasite replication by producing IFN-γ and developing cytotoxic activity against infected cells, thus preventing reactivation of the infection [22, 23].

Current vaccine candidate for *Toxoplasma gondii*

In the past two decades, diverse arrays of vaccine strategies have been developed. These include live attenuated vaccines, killed vaccines, recombinant proteins, subunit vaccines , viral vectors and DNA vaccines[24]. Significant progress has been made in developing vaccine against *T. gondii*. Currently, there is only one commercial vaccine, Toxovax® (live attenuated tachyzoite of strain S48), has been licensed for controlling abortion in sheep. However, this vaccine has not gained widely acceptance due to its short shelf life, high cost, and pathogenic and virulent nature for humans, as it is a live-attenuated vaccine [25]. The focus of candidate vaccine antigens from *T. gondii* has mainly focused on surface antigens (SAG), rhoptries antigens (ROP), microneme antigens (MIC), and dense granule antigens (GRA) [26].

Recombinant Proteins as candidate against toxoplasmosis

These vaccines use specific proteins from *T. gondii* to stimulate an immune response. Surface antigens (SAG), Rhoptries antigens (ROP), microneme antigens (MIC), and dense granule antigens (GRA) are commonly targeted.

***Toxoplasma gondii* surface antigen as a vaccine candidate against toxoplasmosis**

The surface antigen of *T. gondii* is the first component to come into contact with the host cells. There are five proteins in the super family of the surface antigen glycoprotein (SAG) of *T. gondii*, including SAG1, SAG2, p23, p35, and SAG3 [27]. *Toxoplasma gondii* SAGs are involved in host-cell attachment and the activation of the host immune response [28].

SAG1 protein is the main surface antigen of *T. gondii* tachyzoite, is highly conserved in *T. gondii* strains, and enhances high antibody levels in humans [29]. This antigen is highly immunogenic in humans and also capable of enhancing a strong humoral response in immunized mice [30].

In recent years, significant progress has been made in the identification of vaccine candidates that can induce a protective immune response. Most of the work has focused on the surface antigens of tachyzoite. SAG1, SAG2, and SAG2, and SAG3 are major surface antigens of tachyzoite; amongst them, SAG1 is one of the major vaccine candidates [29]. Early studies on the development of vaccines against *T. gondii* focused on SAG 1. These studies have used natural SAG 1 protein purified from tachyzoite [30], recombinant SAG 1 produced in *Escherichia coli and* yeast [29], as well as SAG 1--derived peptides [31].

Studies by Ke-Yi Liu *et al.* showed that SAG 1 induce dominant a dominant antibody (IgG and IgM) response and a strong TH 1 T cell response characterized by high titer IFN γ production during BALB/C mice immunized with recombinant SAG1 (r 1 (r SAG 1) and challenged with a lethal strain of *T. gondii* RH. The immune protection induced by SAG 1 against *T.gondii* is regulated by both hormonal and cell--mediated responses [32] .

Intranasal immunization of CBA/J mice with natural SAG-1 protein combined with cholera toxin as an adjuvant effectively prevented brain cyst formation following oral challenge with *T. gondii*. Both systemic and mucosal antibody responses, as well as systemic cellular immunity, were observed in response to SAG 1 and cholera toxin [33] .

Immunization mice with rSAG1 encapsulated in a delivery system of PLG polymers-formed microparticles (PLG-rSAG1) induced significant, long-lasting SAG1-specific humoral and cell-mediated immune responses. This resulted

in an 80% survival rate compared to 20% protection in the group immunized with rSAG alone, following a lethal subcutaneous tachyzoite challenge [34] .

Recently, studies have shown that virus-based vaccines against toxoplasmosis can mimic the intracellular niche of the parasite. Following the invasion of the host cells by live or attenuated vectors, recombinant vaccines can induce both cellular and humoral immune responses. Adenoviral vectors, which can stimulate pro-inflammatory cytokines have been used [35]. The expression of the *T. gondii* SAG1 gene in adenoviral vectors induces protective immunity that depends on Myeloid Differentiation Factor 88 [36]. Immunization with recombinant influenza viruses encoding *T. gondii* SAG2, followed by a recombinant adenovirus expressing the same antigen, has been demonstrated to boost both cellular and humoral immune responses ,leading to an 85% reduction in parasite burden in mice [37].

Additionally, cross-immunity between *Neosporacaninum* and *T.gondii* could be exploited in heterogonous vaccination strategies based on the close relationship between the two pathogens. Zhang et al. constructed an *N. caninum* live vaccine vector stably expressing the TgSAG1 gene. Mice immunized with this live vaccine elicited Th1-dominant immune responses and were protected from lethal challenge with bradyzoites of *T. gondii* [38].

Administering the SAG-1 antigen in combination with various cytokines, such as IL-2, IL-4, IL-7, IL-15, IL-18, and IL-21, can significantly stimulate cellular responses, thereby enhancing a stronger protective immune response against *T. gondii* after immunization. Additionally ,Co-delivery of an IL-12 plasmid with a single or a mixture of *T. gondii* antigens supports the development of the Th1 immune response, thereby increasing survival rates [39].

Marine IL-18 can activate NK cells and synergize with IL-12 to stimulate NK cell production of IFN-γ. Co-delivery of this cytokine with SAG1 significantly increased the survival rate to 60% , compared with SAG1 alone [40].

Surface antigen glycoprotein 1 (SAG1) affinity purified from the *T. gondii* RH strain, combined with adjuvant, provided significant reductions in brain cyst loads and high survival rates in mice [41]. Notably, the highest protection achieved with this native antigen was obtained with intranasal delivered SAG1: 90% survival and no brain cysts in survivors when administered with the adjuvant QuilA, and an 85% reduction in brain cyst load when adjuvant with Cholera Toxin [42]. Oral immunization SAG1 expressed in tobacco chloroplasts using the 90 KDa heat shock protein of *Leshimania infantum (LiHsp80*) as a carrier for a SAG1 antigen elicited SAG1-specific antibodies and reduced cyst burden (Table 1) [43].

Rhoptries antigens as a vaccine candidate against toxoplasmosis

Rhoptries antigens are secreted by Rhoptries, which is a unique apical secretary organelle in all Apicomplexa parasites. Rhoptries antigens are involved in an active parasite's penetration into the host cell. Due to the key biological role of Rhoptries, proteins have recently become vaccine candidates for the prevention of toxoplasmosis [44].

There are several Rhoptries proteins (ROP), and the most abundant is the ROP2-related family, which includes ROP2, ROP3, ROP4, ROP7, and ROP8. ROP2 is thought to serve as the molecular link between host cell mitochondria and the parasitophorous vacuole membrane [45]. A study using ROP2-depleted parasites observed that ROP2 was essential for multiplication and invasion of the parasite. ROP2 was recognized by a human T cell clone and isolated from an immune donor. It is specific for the parasite protein and produces high levels of IFN-γ. Additionally, ROP2 has been observed in all stages of the parasite .For these reasons, ROP2 was identified as a candidate vaccine, and plasmids encoding the ROP2 antigen have been used to vaccinate mice [46]. Several Rhoptries proteins of *T. gondii* have been tested as vaccines. Garcia *et al.,* used crude native Rhoptries antigens combined in immune stimulating complexes (ISCOMs) to protect pigs against an infection with the oocyst of the *T. gondii* VEG strain (type III). The subcutaneous immunization procedure led to partial protection against cyst formation in the brain and muscles during chronic infection (as confirmed by bioassay in mice), but it was not protective against acute infection, likely because of the lack of intestinal immunity after subcutaneous immunization [35].

A study by Da Cunha *et al.* showed that crude Rhoptries proteins of *T. gondii* plus QuilA, administered by the intranasal, were used to evaluate the cellular and humoral immune responses and brain cyst burden in pigs. Immunization-induced humoral and cellular immune responses and the protective efficacy were 41.6% and 6.5% for crude ROPs with and without QuilA, respectively. This vaccine can induce significant protection against brain cyst formation [47].

Intramuscular immunization of Kunming mice with the plasmid construct pVAX-ROP18 elicited specific humoral and Th1-type cellular immune responses, enhancing the activation of CD8+ and CD4+ T cells in the spleen. This immunization significantly increased survival time compared to control mice when challenged with the *T. gondii* RH strain [48]. In a study done by Qu et al. [49] the same Rhoptries antigen, ROP18, was examined. This study showed

that recombinant protein ROP18, co-administered with ginsenoside, elicited a strong humeral and cellular immune response in ICR mice challenged with the RH strain tachyzoite, resulting in increased survival time. The antibody levels were dose-dependent: higher vaccination dosage enhanced immunogenicity [50].

Rhoptries protein 9 (ROP9) is a soluble Rhoptries protein that is expressed only in the tachyzoite stage and might be involved in the early stages of invasion. The protein contains putative B cell epitopes and triggers an exclusive CD4+ T cell response [35] .

ROP9 was evaluated by Chen et al. [51]. PVAX-ROP9-vaccinated Kunming mice, which were challenged by a highly virulent *T. gondii* RH strain, elicited an effective humeral and cellular Th1 type immune response and prolonged survival time against chronic toxoplasmosis. Although it produced partial protection against acute toxoplasmosis [52].

Another Rhoptries protein vaccine candidate of *T.gondii* is ROP17, which was studied in 2014 by Hai-Long Wang. This study showed that Rh strain tachyzoite-challenged BALB/c mice vaccinated intranasal with rTg ROP17 produced high levels of IgG1/IgG2a and Th1 immune responses (IFN γ and IL-2), and also produced anti-TgROP17-specific secretary IgA. The vaccinated mice reduced brain and liver parasite burdens by 59% and 49%, respectively. This Rhoptries protein kinase is involved in modulating host cell signaling pathways to facilitate parasite survival and replication. ROP18 another Rhoptries protein kinase, ROP18 is crucial for virulence. It helps the parasite evade the host's immune response by phosphorylating and inactivating host proteins that would otherwise target the parasite for destruction [47].

Microneme antigens as a vaccine candidate against toxoplasmosis

Microneme proteins are crucial in the early stages of *T. gondii* adhesion and host cell invasion [53]. Recently, these proteins have gained attention as potential vaccine candidates. Immunizing Mice with MIC 1 and MIC 4 proteins purified from tachyzoite resulted in a Th1 immune response and increased protection levels [54]. MIC 3, a significant adhesion protein of *T.gondii*, has also shown promise as a vaccine candidate. Mice (CBA/J) immunized with plasmid DNA encoding the immature form of the MIC 3 protein displayed significant protection against oral challenge with *T.gondii* cysts, showing fewer brain cysts than the control mice [55].

Moreover, MIC1 and MIC4 proteins purified from parasite extracts have been found to reduce brain cyst numbers, leading to an 80% survival rate and a 68% reduction in brain cyst load in C57BL/6 mice after challenge with *T. gondii* ME49 [56].

MIC 13 is also another vaccine candidate of the miceronems protein of *Toxoplasma gondii*, which plays an important role in Toxoplasma dissemination because it has three micronme adhesive repeat domains, which act as an important determinant in host cell recognition by binding salivated glycol conjugates on the gut epithelium [57].

Yuan et al. [58] showed that vaccination with pVAX-TgMIC13 can enhance specific cellular and humoral immune responses in murine models, as shown by a significant IgG response and Th1 type cytokine production, and partial protective immunity against acute and chronic infection by *T. gondii* PRU and RH strains. This was demonstrated by a significantly increased survival time and 57.14% cyst reduction compared with the mice in the control group (Table 1) [59].

Dense granule antigens as a vaccine candidate against toxoplasmosis

The dense granule is a secretary vesicular organelle that expresses proteins in the tachyzoite, bradyzoites, and sporozoites stages of *T. gondii*. These proteins (GRA) induce a strong antibody response during acute infection. Thus, GRA proteins have been considered as vaccine candidates for the prevention and control of toxoplasmosis [60]. Importantly, GRA7 has the ability to stimulate significant cellular and humoral immune responses against *T. gondii* [61]. A recombinant prokaryotic plasmid pET30-GRA7 and eukaryotic plasmid pEGFP-GRA7 performed in BALB/c mice with an adjuvant of phosphate- buffered saline induces both a humoral and cellular immune responses against *T. gondii*. Enhancement of protective immune responses induced by *T.gondii* dense granule antigen 7 (GRA7) against toxoplasmosis in mice using a prime-boost vaccination strategy [62].

A study using recombinant GRA2 and GRA6, with monophosphoryl A (MPL) as an adjuvant, found a significant reduction in the number of brain cysts in GRA2, but not in GRA6, immunized CBA/J mice challenged with *T. gondii*. The combination of both antigens did not lead to enhanced protection [63]

Recently, two studies using adjuvant mixtures of recombinant proteins achieved a high level of protection against chronic toxoplasmosis. A mixture of SAG1, GRA1, and MAG1 adjuvant with Freund's complete adjuvant reduced brain cyst burden by 89% in BALB/c mice [64]. Additionally, a mixture of GERBU adjuvant GRA7 and a MIC2-MIC3-SAG1 chimeric protein provided a 79% reduction in brain cysts in outbred SWISS mice following challenge with *T. gondii* [65].

Table 1. Immunization Experiments originated from surface antigens, Granule, Microneme and Rhoptries.

Category	Antigen	Animal model	Route of immunization/ Adjuvant	Challenge	Result	Ref
ROP	pROP9	BALB/c mice	IM	Tachyzoite of RH strain	High level of specific anti *T. gondii* anti body (IgG1/IgG2a) Induced Th 1 cellular response(INF γ/IL-2)	[51]
	rROP 17	BALB/c mice	In	Tachyzoite of RH strain	Reduce liver and brain parasite burden 59%and 49%,respectively High level of IgG1/IgG2a and IFNγ and IL-2 Strong mucosal immune response(IgA secretion)	[50]
	rROP18	ICR mice	SC / ginsenoside Re	Tachyzoite of RH strain	strong humeral and cellular immune response increase survival time	[49]
MIC	pMIC13	Kunming mice	IM	Tachyzoites of RH strain Cyst of PRU strain	Reduce brain cyst burden increase survival time High level of cellular and humeral immune production	[58]
SAG	rSAG1	C57BL/6 mice	Oral/chloroplast of tobacco	Cyst of ME49 strain	57% reduction of cyst A significant increase of SAG1-specific IgG antibody	[66]
Mixed antigens	rROP2+rGRA4+rSAG1	BALB/c mice	SC/Freud's	Tissue cyst of DX *T.gondii*	Partial protection against chronic toxoplasmosis 46% reduce brain cyst High level of antigen specific IgG1/IgG2a	[67]
	rROP2+rROP4+rGRA4	BALB/c mice	SC/Freud's	Tissue cyst of DX *T.gondii*	Partial protection 84% reduce brain cyst High level of antigen specific IgG1/IgG2a	[67]
	rROP2+rROP4+rSAG1	BALB/c mice	SC/Freud's	Tissue cyst of DX *T.gondii*	Partial protection 77% reduce brain cyst High level of antigen specific IgG1/IgG2a	[67]
	rROP2+rROP4+rGRA4	C3H/HeJ and C57BL/6	SC/Freud's	Tissue cyst of low virulence DX *T.gondii* strain	Reduce 59% and 41% brain cyst load in C3H/HeJ and C57BL/6 mice, respectively	[68]
	rROP2+rROP4+rSAG1	C3H/HeJ and C57BL/6	SC/Freud's	Tissue cyst of low virulence DX *T.gondii* strain	Reduce 71% and 90% brain cyst load in C3H/HeJ and C57BL/6 mice, respectively	[68]

GRA: Dense granule antigens; Im: Intramuscular; in.: Intranasal; MIC: microneme antigens; PLG: poly (lactide-co-glycoside); Re: ginsenoside Re; ROP: Rhoptries antigens; SAG: surface antigen; SC.: subcutaneous.

DNA as a vaccine candidate against toxoplasmosis

DNA-based vaccines have been used as potential candidates for protection against parasitic infections because of their capacity to induce both cellular and humoral immunity. The advantages of DNA-based vaccines are generally safe and non-toxic [69].

DNA vaccinations against toxoplasmosis would generally induce a Th1 immune response with an increased production of the inflammatory cytokines IFN-γ and IL-2 to limit infection. DNA vaccinations are also effective at inducing the activation and proliferation of CD4+ and CD8+ T cells, along with eliciting specific antibodies essential for the control of chronic infection [70].

DNA vaccines are considered an alternative approach to live, attenuated vaccines because they can enhance long-lived immune responses in animals [71]. Moreover, these vaccines have been seen to be safe and effective in controlling *T. gondii* infection. Cellular immune responses generated through immunization are particularly important for combating *T.gondii*, and intramuscular DNA vaccines are known to induce both cellular and humoral immunity [42] .

DNA vaccines generate only weak immune responses in the absence of suitable adjutants. Various efforts have been made to elicit immune responses, usually involving the co-expression of cytokine genes, BCG, or co-stimulator genes [72] .

A recombinant plasmid pc DNA/Tg SAG1 vaccine with IL-18 as an adjuvant. Female BALB/c mice challenged interpersonally with highly virulent RH strain tachyzoite of *T.gondii* immunized with pcDNA/TgSAG1 with and without pVAX/mIL-18 showed that mice that were vaccinated with a co-injection of pVAX/mIL-18 produced a higher level of IFN γ and IL-2 compared with pcDNA/TgSAG1 alone. Additionally, Th2 type cytokine levels, i.e., IL-4 and IL-10, decreased in the presence of IL-18. As described above, pcDNA/TgSAG1 with adjuvant IL-18 was a vaccine candidate to induce a high level of Th1-type cytokines and partial protection against *T.gondii* [73, 74].

A study combining the two bradyzoites antigens BAG1 and MAG1 in a cocktail DNA vaccine also found a significant reduction in the number of brain cysts (62%), but no sterile immunity (75%). A similar study, using a combination of antigens delivered as plasmids coding for regions of microneme proteins including MIC2, MIC3, MIC4, M2AP, and AMA1, resulted in a significant reduction (84%) of the number of cysts but not sterile immunity [75] .

Other studies have combined *T. gondii* antigens on a single DNA vaccine plasmid. Chimeric SAG1-ROP2 and multi-antigenic DNA vaccines containing SAG1, ROP2, and GRA2 showed significant protection levels against acute toxoplasmosis in BALB/c mice. The addition of IL-12 (but not cholera toxin) as a genetic adjuvant could enhance the protective Th1 response and protection [76].

DNA vaccine encoding the target antigen of *T. gondii*, SAG1, delivered by attenuated *S.typhimurium* is a simple and potent vaccine that elicits a higher antibody response and provides significant protection against *T. gondii* infection. A higher amount of *T.gondii*-specific IgG and IgM subclasses and IFN γ were detected in immunized mice. Mice orally immunized with ZJ111/pcDNA-SAG1 were challenged with 500 tachyzoites of *T. gondii,* resulting in a survival rate of 20% higher than the control [77] .

DNA vaccine containing sporo SAG and the anti-apoptotic Bcl-XL gene was generated to increase the antigen-specific CD8+ T lymphocyte response. This study evaluates for the first time the ability of sporo SAG to induce a protective immune response using a DNA vaccine. The mice vaccinated with the sporo SAG/Bcl-XL DNA vaccine induced a high IgG and CD8+ T lymphocyte excreting IFNγ response against sporo SAG [78].

A recent study explored the potential cocktail DNA vaccine encoding GRA1 and GRA7 to provide high-level protection against both acute and latent *T. gondii* infection. The *T. gondii* GRA1 DNA vaccine can induce CD8+ T cell proliferation in vaccinated mice, offering protective against acute disease. The production of IFN-γ , by GRA7-specific T cells is necessary for high protection against latent toxoplasmosis [30].

Another study conducted by Wang and Yin [55] showed that DNA and *Adenovirus* vector vaccines encoding multi stage antigens elicited a mogreaterh-1 type-immunity. Vaccines prepared from seven antigens, such as SAG3, ROP18, MIC6, GRA7, MAG1, BAG1 and SPA expressed in tachyzoite, bradyzoites, sporozoites, and immunized with DNA and *Adenovirus* in BALB/c mice, induced a high level of IgG2a and Th-1 cytokines, such as IL-2 and IFN-γ, compared with the control group [37].

Multi antigenic (cocktail) vaccines

Multi-antigen vaccines expressing different proteins in several stages of the parasite increase protective efficacy compared with single-antigen vaccines. Recent studies showed that a cocktail surface and secretary antigens were prepared as a vaccine candidate for *T. gondii*. The rROP2+ rROP4+ rGAR4 and rROP2+ rROP4+ rSAG1 vaccines

were tested on two mouse strains, C3H/HeJ and C57BL/6, which are highly and intermediately susceptible to *T. gondii* invasion, respectively. The rROP2+ rROP4+ rGAR4 and rROP2+ rROP4+ rSAG1 vaccines resulted in a 59% and 71% reduction in brain cyst load C3H/HeJ mice, respectively. C57BL/6 mice, these vaccines reduced brain cyst loads by 41% and 90%, respectively [33].

Followed this study, others also demonstrated that the three mixtures of recombinant Toxoplasma antigens (rROP2 + rGAR4 + rSAG1, rROP2 + rROP4 + rGAR4, and rROP2 + rROP4 + rSAG1) with Freud's adjutants, when administered subcutaneously in BALB/c mice and challenged the DX strain brain cyst of *T. gondii*, induced a significant reduction in brain cyst number. The reductions were 46%, 84%, and 77%, respectively, compared to control PBS (phosphate-buffered saline)-injected BALB/c mice. Thus, cocktails of secretary recombinant Rhoptries proteins rROP2 and rROP4 co-administered with surface rSAG1 or secretary rGRA4 antigen provide strong partial protection against chronic Toxoplasma invasion [79].

A recent study discussed that cocktailed vaccine candidates SAG1 and ROP2, when immunized in mice induce, Th1-type cellular responses, which are measured by IgG, IFN-γ, TNF-γ, and IL-12, and also increase survival time against the *T.gondii* RH strain compared to those immunized with a single-gene vaccine [34].

A vaccine cocktail of SAG1 and MIC3 expressed in a pseudo-type *baculo*virus vector induced significantly better protection compared with a single-gene vaccine. Studies concluded that the recombinant pseudo-type *baculo*virus cocktails of SAG1 and MIC3 induced better immunogenicity than the plasmid DNA vaccines. Co-delivery of SAG1+MIC3 was also found to enhance the protective efficacy [80].

A DNA vaccine encoding *T. gondii* superoxide dismutase (Tg SOD) is a potential vaccine candidate agonist for toxoplasmosis. *T.gondii* superoxide dismutase might participate in affecting the intracellular growth of both tachyzoite and bradyzoites forms. A DNA vaccine encoding SOD could trigger strong humoral and cellular immune responses and elicit partial protective immunity against acute *T. gondii* infection in the murine model (Table 2) [81].

CHALLENGES AND FUTURE DIRECTION OF VACCINES AGAINST *T. gondii*

In recent years significant progress has been made in the search for a vaccine to prevent toxoplasmosis. However, developing a vaccine against *T. gondii* presents several challenges. Killed, inactivated, and crude antigen vaccines for *T. gondii* are not effective. In contrast, attenuated live vaccines elicit a highly mobilized CD8+ T-cell response, crucial for clearing intracellular infection [82]. However, the immunogenicity of live vaccines is affected by their degree of attenuation, which limits their shelf life. Additionally ,there are safety concerns, as attenuated vaccines carry the risk of reverting to a pathogenic strain [83]. Subunit vaccines could potentially cause allergic reactions since the protein is expressed within another organism. Moreover, subunit vaccines tend to lack immunogenicity and therefore require suitable adjutants to enhance vaccine potency. DNA vaccines have certain advantages; they can elicit both cellular and humoral immune responses. However, DNA vaccines generate only weak immune responses when used in humans [84].

Due to the complex life cycle of *T. gondii*, which involves multiple hosts with diverse protein forms and various invasion pathways, several candidate antigens have been identified. However, those capable of inducing strong and long-lasting protective immunity are limited. Accumulating evidence suggests that vaccination with stage-specific antigens leads to stage-limited protection against *T. gondii* [30, 35].

Future studies should focus on developing effective vaccines by selecting target antigens and presenting them to the immune system through optimal delivery strategies to stimulate appropriate protective immunity. Moreover, vaccines against toxoplasmosis should include different antigens expressed at all stages of the parasite, combined with an adequate adjuvant and appropriate delivery strategy. There should be increased awareness of the importance of selecting the right vaccine candidates, as well as choosing suitable adjutants and delivery methods. Future studies should also consider the mechanisms of host cell invasion and the immune and pathogenic responses associated with *T. gondii*, as well as the action of vaccines against toxoplasmosis.

Table 2. Examples of immunization with DNA vaccines against *T.gondii*

Antigen	Adjutants/ Antigen delivery	Animal	Challenge	Result	Ref.
ROP9	Im	Kunming mouse	Tachyzoite, RH strain	Increased survival time (12.9±2.9 days)	[51]
ROP8	Im	BALB/c mouse	Tachyzoite, RH strain	Increased survival rate (50%)	[85]
ROP16	Im	BALB/c mouse	Tachyzoite, RH strain	Educed humeral and cellular response. Increase survival time	[86]
SAG1+SAG3	CT/im	BALB/c mouse	Tachyzoite, RH strain	Increase survival rate with CT adjuvant (40%). Increase secretion of IFNγ and high lymphocyte proliferation	[87]
SAG1	Im	BALB/c mouse	Tachyzoite, RH strain	Increase survival time (7.7 ± 2.5 day)	[88]
GRA6	LMS/ im	BALB/c mouse	Tachyzoite, RH strain	Partial protection, higher survival rate with LMS than without adjuvant and control group 53.3, 40% and 0%, respectively. No detectable parasites in brain, liver and spleen of immunized groups	[89]
MIC13	Im	Kunming mouse	Acute: tachyzoite, RH strain Chronic: tissue cyst, PRU strain	RH strain: Increase survival time (21.3±11.3 days) PRU strain: reduction in brain tissue cyst load (57%)	[58]
MIC8	IL-21+ IL-15/ im	Kunming mouse	Acute: tachyzoite, RH strain Chronic: tissue cyst, PRU strain	RH strain challenge: Increased survival time (16.2±1.3 days) PRU strain challenge: Reduction in brain tissue cysts load (63.8%)	[90]
MIC6	Im	Kunming mouse	Tachyzoite, RH strain	Cellular and humeral immune response. Increase survival time.	[91]
TgCDPK3	Im	Kunming mouse	Acute: tachyzoite, RH strain Chronic: tissue cyst, PRU strain	Increase survival time (13.5±4.9 days). Reduce brain tissue cysts (54%)	[92]
TgCDPK5	Im	Kunming mouse	Acute: tachyzoite, RH strain Chronic: tissue cyst, PRU strain	Increase survival time (8.7±4.3 days). Reduce brain tissue cysts (40%)	[93]

IFNγ =Interferonγ ROP= Rhoptries antigen; SAG= surface antigen; GAR= dense granule antigen; MIC= micronme antigen; TgCDPK= *T. gondii* calcium-dependent protein kinase; im, intramuscular; IL= interleukin; CT= cholera toxin; LMS= levamisole

CONCLUSIONS AND RECOMMENDATIONS

There is no available drug for the treatment of *T. gondii* tissue cysts in humans or animals, making the development of a vaccine critical strategy for disease control. Vaccine candidates' of recombinant proteins primary target surface antigen (SAG), Rhoptries antigen (ROP), miceronems antigen (MIC), and dense granule antigen (GRA). DNA vaccines offer distinct advantages by eliciting both cellular and humoral immune responses, with the CD8+ T cell response playing a key role in immunity against *T.gondii*. MultiAntigenic (cocktail) vaccines use viruses to deliver *T. gondii* antigens to the host's immune system, thereby stimulating a protective immune response. Despite efforts, existing vaccines provide only partial protection, highlighting the need for more effective solutions. To develop successful vaccines against *T. gondii,* it is essential to design strategies that block critical stages of the parasite's life cycle or mimic the host's immune response. Current research indicates that multi-antigenic formulations offer better protection than single-subunit vaccines. Future vaccine development should prioritize enhancing protein expression, co-expressing cytokines, and utilizing molecular adjutants. Combining multiple antigens with adjutants or cytokines could improve protective outcomes and bring us closer to achieving sterile immunity. Ongoing research and development of multi-antigenic vaccines are essential to boost the efficacy and potency of toxoplasmosis vaccines. Emphasis should be placed on multi-antigenic formulations and the co-delivery of cytokines and adjutants to enhance vaccine effectiveness and progress towards sterile immunity.

DECLARATIONS

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Authors' contributions

Eden Woldegerima: designed and wrote the review; Fasika Getachew, Meseret Misganaw: critically read and modified the review; Eden Woldegerima , Debaka Belete and Yohannes Mesfin performed literature revision and took care of the editing of the review; Tekeba Sissy and Nega Berhane perform final revision.

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Ethical approval

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