Vaccination Strategies for Toxoplasmosis

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Abstract

Toxoplasma gondii is a common intracellular protozoan that has been found in about one-third of the world's population since its discovery in 1908. *T. gondii* has a high seroprevalence globally because of its complicated life cycle, which allows it to withstand stress and spread readily among a wide range of hosts. Toxoplasmosis is still one of the most common opportunistic neurological illnesses linked to HIV. From the well-known DNA vaccines to the more conventional inactivated whole-*T. gondii* vaccines, this study provides a thorough summary of several immunization strategies. The difficulties in creating these vaccines, choosing adjuvants and delivery systems, immunization strategies, and creating research models are discussed in great detail. Here, we also discuss the most recent and potential improvement techniques that can be used to overcome the obstacles in creating a preventive vaccination against *T. gondii*.

Keywords: *Toxoplasma gondii*, Vaccination, Life cycle, Strategies, Immune Response

Cite this Article as: Javed G, Seyidli M, Zhu Z, Farooqi SH and Nisa QU, 2025. Vaccination strategies for toxoplasmosis. In: Kun Li (ed), Protozoan Zoonoses: Advances in the Diagnosis, Prevention, and Treatment of Cryptosporidiosis and Toxoplasmosis. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 74-80. https://doi.org/10.47278/book.HH/2025.336

A Publication of Unique Scientific Publishers

Chapter No: 25-11

Received: 25-Feb-2025
Revised: 13-Apr-2025
Accepted: 12-May-2025

Introduction

The obligatory intracellular parasite *T. gondii* belongs to the Apicomplexan family. The microorganism has established itself as a parasite in one-third of all humans throughout the globe. The parasite shows ability to infect all cells with a nucleus which explains its wide host range. The parasite demonstrates a wide capability to infect all warm-blooded mammals including humans. Main *T. gondii* isolates originate from North America together with Europe. The parasite strains belong to three main clonal groups named types I II and III. The varieties of *T. gondii* genetic strains tend to show restricted patterns of diversity (Howe & Sibley, 1995). Research indicates that the Felidae family stands as *T. gondii*'s principle host for sexual reproduction. The parasites of *T. gondii* divide asexually across all mammalian species together with their definitive hosting populations (Hutchinson, 1966). *T. gondii* executes its life cycle through three distinctive phases starting from tachyzoite ending at sporozoite with a stopover in bradyzoite. When conditions become acidic (pH 6.6) and basic (pH 8.0–8.2) or temperature reaches 40 C or if the environment includes sodium arsenite the fast-replicating tachyzoites transform into the dormant bradyzoites. The parasite manifests under specific situations such as pH conditions of 6.6 and 8.0–8.2 alongside temperature exposure to 40 C and contact with sodium arsenite (Soete et al., 1993; Soete et al., 1994).

The multiplication rate of Bradyzoites is slow because they remain in a chemical state of dormancy. The life-long existence of *T. gondii* inside human body occurs when bradyzoites develop within host cells to form cysts that become dormant yet retain asymptomatic status as shown in figure 1. Recrudescent infection develops in patients whose immune system is compromised thus forcing the protozoan to convert from bradyzoites into tachyzoites (Lyons et al., 2002).

Bradyzoites reach the sexual stage of merozoites through all five schizont stages in the feline intestine within 2 days. Merozoites start doubling themselves while progressing to become macrogametes. After microgametes and macrogametes develop through sexual reproduction an oocyst forms with either male or female genetics. The oocysts that develop within the feline feces obtain their protective oocyst wall before completing their sporulation to sporozoites (Ferguson et al., 1974).

There exist three fundamental ways for *T. gondii* to spread:

(1) Foodborne transmission through eating raw meat tissues or drinking water containing oocysts as shown in Fig. 1.

(2) Zoonotic transmission through accidental consumption of oocysts upon contact with infected hosts or contaminated settings.

(3) Congenital transmission through placental passage during pregnancy (Dubey & Jones, 2008).

The simple nature of *T. gondii* transmission leads to high seroprevalence rates reaching 90% in particular regions of Europe and South America. Results show that toxoplasmosis affects 22.5% of people who are 12 years or older in the United States population (Flegr et al., 2014). Tissue cysts of *T. gondii* in immunocompromised hosts become active which leads to different medical complications although most potential infections remain asymptomatic due to effective immune regulation. The occurrence of toxoplasma encephalitis becomes more common among

AIDS/HIV patients. Pregnant individuals face two risks because *T. gondii* can cross through the placenta to infect the fetus or lead to pregnancy termination. Diagnosis of congenital infection occurs with infants who developed the infection before birth (Wang et al., 2017a). The disease appears in different ways through chorioretinitis and it leads to calcification and hydrocephalus. Research by meta-analysts identified a positive relationship between *T. gondii* infection and psychotic symptoms along with psychotic disorders including schizophrenia and bipolar disorder (Sutterland et al., 2015).

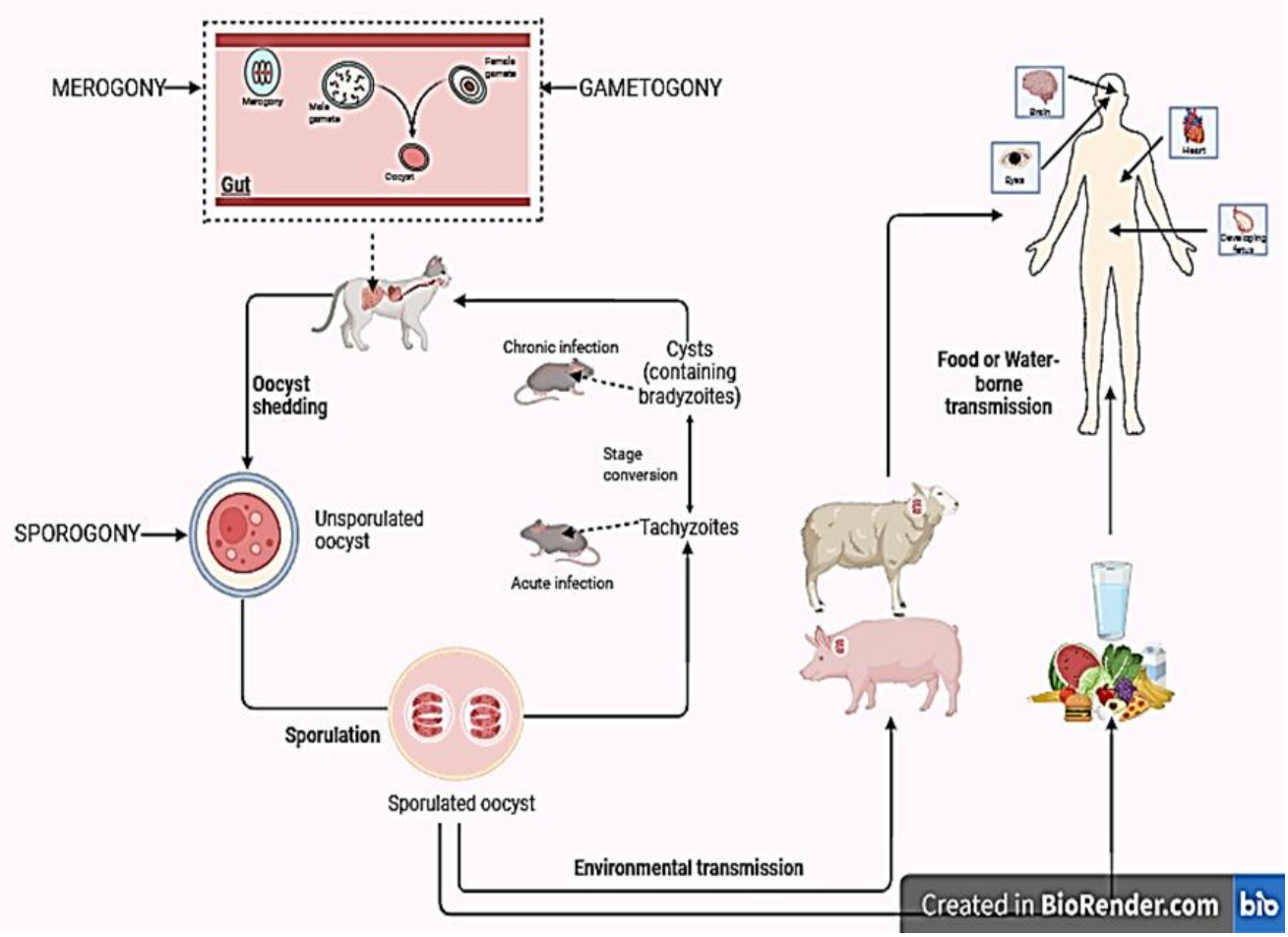

Fig. 1: This depicts the life cycle of a cyst forming Toxoplasma.

Medical research shows that the combination of PS represents an established clinical approach for treating active toxoplasmosis in humans because it targets *T. gondii* folate metabolism followed by preventing nucleic acids synthesis. PS use leads to severe adverse effects which include lowered platelet counts combined with neutropenia as well as hypersensitivity reactions. The teratogenic characteristic of pyrimethamine causes doctors to give spiramycin to pregnant patients because this treatment displays minimal harm to developing foetal tissues (Dunay et al., 2018). The placenta prevents spiramycin from reaching the fetus which means the drug works to stop vertical *T. gondii* transmission but cannot treat fetal *T. gondii* infection. Medical therapists currently employ drugs that only destroy tachyzoites but fail to eliminate tissue cysts in patients with chronic infection. The slow rate of DNA synthesis in tissue cyst bradyzoites prevents formation of folate by anti-folate drugs. Livestock infectious disease control depends on the commercially available Toxovax vaccine as the one and only live attenuated *T. gondii* vaccine for sheep and goats' abortion prevention. The vaccine exists for use in the UK along with New Zealand and France and Ireland (Garcia et al., 2014). The parasite elimination with this medication is impossible and it poses dangers for human use because it may shift into pathogenic form and trigger iatrogenic infection (Buxton & Innes, 1995).

The flexible adaptation of *T. gondii* allows this successful parasite to transform its life cycle and spread asexually and sexually through various pathogens across the globe. Active immunization provides the best possible method for activating host defenses against *T. gondii* infections that extends over long periods. Research has shown continuous advancement in *T. gondii* vaccine development and this study reveals novel approaches among current studies. The chapter includes future guidance regarding *T. gondii* vaccine development and different strategies to improve vaccine immunity in response to existing constraints.

2. Recent Developments in Vaccines against Toxoplasmosis

Scientific research on creating *T. gondii* vaccines led to multiple successful preclinical tests. There are four main strategies which will be presented in detail as vaccine developments for *T. gondii* in the following sections:

2.1. Nucleic Acid Vaccines

The benefits of DNA vaccines include security together with quick manufacturing processes and stable preservation capabilities alongside their effectiveness toward stimulating both cellular and humoral immunity. The testing of more than fifty *T. gondii* DNA vaccine candidates demonstrated good protection through different challenge tests during the past five years (Alarcon et al., 1999). Researchers developed multiple vaccine enhancement methods which included (i) adjuvant utilization for improved immunity strength and (ii) bacterial or viral carriers for better antigen delivery and (iii) using multiple antigens for protecting against multiple strains and (iv) bioinformatics-based antigen assessment of candidate genes with (v) heterologous prime-boost vaccination strategies. The inducement of humoral and cellular immunity through two vaccination approaches can be achieved by utilizing heterologous prime-boost regimen and gene-based strategies. The DNA candidate vaccines demonstrated protection with survival longer than 30 days against type I tachyzoites or demonstrated more than a 70% reduction in type II cysts. The most studied vaccine constructs using DNA come from Rhoptry proteins (ROPs). The strong immune response might have been triggered by ROPs because they take charge of host cell invasion. Among ROP16 and ROP18 proteins these two have shown the most effective defense against Type I-RH. Active immunization employing canine adenovirus type 2 (CAV2) revealed strong proinflammatory cytokine production of IFN-c and IL-2 during Th1 response as it delivered both antigens (Li et al., 2015; Li et al., 2016).

Researchers have examined RNA-based vaccines that need an efficient delivery system as an alternative for *T. gondii* management. The first study using modified dendrimer nanoparticles (MDNPs) as mRNA replicons to deliver six antigens of *T. gondii* was published by Chahal et al. (2016). A shipment technology known as MDNP comprises synthetic components which assemble replicons with ionizable delivery agents together with lipid-attached polyethylene glycol to maintain the stability of exposed mRNA. The nano-delivery system defends *T. gondii* antigens from degradation processes while improving their ability to reach the host recipient. The newly developed platform provided complete protection against Type II PRU strain after a single vaccination. Vaccine manufacturing needs only one week to complete its production process (Chahal et al., 2016). Protection against Type I virulent strains along with Type II avirulent strain cyst number reduction was not included in this study. Luo et al. created a lipid nanoparticle-based self-amplifying RNA vaccine containing *T. gondii* nucleoside triphosphate hydrolase-II (NTPase-II) as the antigen (Yan et al., 2024). Mice that received the vaccination showed substantial defense against Type II PRU strain infection using a 62.1% reduction in brain cyst numbers. The studies establish that *T. gondii* RNA vaccine development shows promise toward creating effective vaccine solutions (Luo et al., 2017).

2.2. Recombinant Protein Vaccines

Recombinant protein vaccines use meticulously purified pathogen epitopes as antigens to develop their defined and highly purified components. The vaccines based on recombinant protein antigens produce outcomes with lower immunogenicity because they maintain less of the pathogen-associated molecular pattern (PAMPs) when compared to whole-pathogen vaccines (Coffman et al., 2010). Antigen degradation through proteolytic activity has the potential to occur within host cells. Due to their protective effects on vaccination potency and resistance to degradation adjuvants are included regularly in recombinant protein vaccines (Skwarczynski & Toth, 2016). Research into *T. gondii* protein vaccines has grown in numbers despite various challenges. Different categories of adjuvants emerged as a response to improve immune responsiveness (Karakavuk et al., 2022). The protein vaccine protections extend past 30 days for type I tachyzoite survival as well as display more than 70% cyst reductions of type II cysts. The rhoptry protein ROP remains the most popular vaccine candidate among DNA vaccines since scientists use it independently or with multiple antigens for maximal protection benefits. The combination of recombinant ROP18 with ROP38 and CDPK6 demonstrates high capability to eliminate Type II PRU brain cysts. Diagnostic vaccine containing recombinant antigen combination reduced brain cysts by more than 70% (rROP18-rROP38) and 50% (rROP18-rCDPK6) when injected alone to laboratory animals (Xu et al., 2015; Zhang et al., 2016). The antigens encapsulated in poly (lactide-co-glycolide) (PLG) resulted in higher percentages 81.3% and 73.6% due to the protective effect and controlled antigen release over time. There exist antigens which demonstrate significant immunogenic potential only after combining them with adjuvants. The combination of porous nanoparticle adjuvant with *T. gondii* antigen lysates achieves brain cyst reduction of 70% in experiments but the individual use of lysates or nanoparticle results in only 20% of cysts reduction (Dimier-Poisson et al., 2015).

2.3. Live Attenuated Vaccines

The immune response generated by live attenuated vaccines derives from target pathogens whose virulence factors were reduced to enable limited replication inside host cells. An attenuated *T. gondii* vaccine presents an ideal solution for toxoplasmosis because it expresses various antigens that match life cycle stages. Most of the live attenuated *T. gondii* vaccines provided complete survival protection for at least 30 days after challenge. Selective gene alteration or disruption becomes possible through availability of the *T. gondii* genome sequence for targeted attenuation purposes (Gigley et al., 2009a). The application of live attenuated vaccines remains restricted because the pathogenic form of the disease might reappear from the weakened vaccine strain. The major targets for attenuating *T. gondii* strains exist within these enzymes. The removal of carbamoyl phosphate synthetase II (CPSII) regulatory enzyme resulted in the creation of a completely harmless *T. gondii* cps1-1 mutant strain with uracil auxotrophic characteristics. Research indicates that the strain creates enduring protection against brain cysts of both the type I RH strain and type II ME49 strain (Gigley et al., 2009b).

The research of Fox and colleagues made sure their targeted gene replacement was efficient by deleting the KU80 gene in *T. gondii* (Fox et al., 2011). KU80 protein works within the *T. gondii*'s non-homologous end-joining DNA repair pathway to initiate high-frequency non-homologous recombinant events which sink the gene targeting method. The KU80-deleted *T. gondii* strain served as the background strain for studies that included disruption of ompdc and disruption also occurred in the de novo pyrimidine biosynthetic pathway. The strain generated complete protection from *T. gondii* acute disease through CD8+ T cell dependent immunity that defended against type I and type II strains of the parasite. Elimination of cyst formation by the type II strain was observed during research (Fox & Bzik, 2015). When combined with KU80

knockout background the *T. gondii* type I strain remained completely protected by a lactate dehydrogenase mutant strain (Abdelbaset et al., 2017). The creation of vaccine strains by deleting *T. gondii* essential genes AMA1 and GRA17 and CDPK2 provides comprehensive protection against *T. gondii* strains of type I and type II (Wang et al., 2017b).

The methods for strain attenuation of *T. gondii* shifted from natural to genetic deletion processes. The 1988 version of Toxovax S48 strain received its attenuation via over 3000 successive lab passages. The spontaneous mutations make the strain vulnerable to develop its pathogenic characteristics back (Wilkins et al., 1988). When compared to the current knockout strains the defined deletions prevent reversion of pathogenicity yet remain relatively safe for human use. They present a level of security that makes them safe for human applications. The safety of the vaccine increases when it contains MIC1- MIC3 double knockout strain because in vivo reversion of double mutants becomes imperceptible (Mévélec et al., 2010).

2.4. Inactivated Whole-Pathogen Vaccines

One of the primary methods for vaccine making involves treating pathogens with chemical and physical treatments for inactivation. The pathogen-destroying efficiency of radiation sterilization makes it possible to eliminate chemical contaminants and effectively reach nucleic acids to cause destruction without altering the surface antigens during the process. The irradiate strains retain their biological features which include cellular structures and DNA and protein synthesis and invasion ability. These vaccine strains need additional immunization boosters together with adjuvants because they lack their original capacity to replicate or infect the body. The vaccination against toxoplasmosis makes use of strains that have been subject to gamma irradiation or UV attenuation processes. The usage of *T. gondii* strain-based vaccinations has decreased because of worries about strain de-virilizations back to its pathogen form (Zorgi et al., 2011).

3. Strategies to Improve Vaccine Immunity in Response to Existing Constraints

3.1. Reasons for Using Efficient Bioinformatics for Antigen Selection

The selection process for antigens represents a vital first decision. The researchers behind *T. gondii* vaccine studies utilized selected antigens because of their involvement in invasion and infection processes (Ayub et al., 2023). The ability to infect hosts at high rates does not reliably predict how well an antigen can activate the immune response. The process of locating and predicting antigen epitopes should be performed using immunoinformatic methodologies because of its crucial importance (Nosrati et al., 2020). The infection agent known as antigen stimulates different types of immune response systems within the host body. Through ToxoDB online database (http://ToxoDB.org) researchers can access genome resources of *T. gondii* strains ME49, GT1, VEG and RH. *T. gondii* sequence data availability enabled reverse vaccinology to become possible by creating a systematic candidate vaccine screening that considers immunogenic potential (Mohammadhasani et al., 2024).

T. gondii vaccine development should include both T-cell epitopes for cytotoxic T lymphocyte (CTL) response stimulation and B-cell epitopes for antibody production. Epitope study for T-cells requires two prediction processes: cytotoxic T lymphocyte (CTL) identification and T helper (Th) cell recognition. The research tools supported by Immune Epitope Database (IEDB) let users forecast the half maximal inhibitory concentration (IC50) values of peptides binding to MHC or HLA molecules (Han et al., 2017). Linear arrangements between T-cell epitopes and MHCs make it possible to precisely model the binding interface of ligands to T-cells. It becomes complicated to identify B-cell epitopes since there are no detectable physicochemical patterns throughout the amino acid sequences for computational prediction (Blythe & Flower, 2005). Some B-cell epitopes modify their structural conformation after binding to their cognate antibody's paratope which makes 3-dimensional (3D) structure-based prediction more complex (Rueckert & Guzmán, 2012). The prediction software I-TASSER and SOPMA generate 3D models for proteins through bioinformatics processes (Song et al., 2017).

3.2. Antigen Expression (Stable and Cross-Protective)

The effectiveness of immune responses depends on obtaining stable antigen production for vaccinated subjects. Research shows that the standard approach in manufacturing *T. gondii* DNA vaccines utilize eukaryotic expression vectors with viral promoters to generate maximum gene expression inside the body. The immediate early-enhancer or promoter activity of Cytomegalovirus (CMV) becomes a standard choice in DNA vaccines since it provides the highest level of transgene expression (Cheng et al., 1993). The expression of transgenic DNA vaccine promoters driven from viral elements becomes inhibited by IFN-ɤ and tumour necrosis factors (TNF-a) output from immune system cells (Vanniasinkam et al., 2006). Analysis of an adenoviral vector showed that CMV promoter activity experienced reduction in reporter gene expression (Sung et al., 2001). The research found that anti-viral properties of specific cytokines operated on promoter activity to control transgene expression levels. Most *T. gondii* DNA vaccine research that implements constitutive promoters might experience promoter activity limitations from high levels of IFN-ɤ that results in unstable antigen expression beyond a certain timeframe. The irregular antigen manifestation combined with expression control mechanisms might contribute to the limited protective success. Eukaryotic promoters like MHC class II which remain resistant to IFN-ɤ regulation should be utilized for antigen expression of *T. gondii* (Vanniasinkam et al., 2006).

The protection levels from *T. gondii* vaccine candidates show improvement through the incorporation of multiple antigens. The vaccines have the capacity to use multiple antigen candidates for developing diverse immune responses. Multiple antigen vaccines duplicate natural infection antigen processing as well as presentation steps to the immune system (Suhrbier, 1997). The efficiency of vaccines can improve through using multiple antigen candidates because different people have varying levels of antigen presentation capacity. Research has brought forth two vaccine approaches which rely on attenuated *Salmonella typhimurium* to transport plasmid-encoding *T. gondii* antigens through the use of single antigens SAG1 and SAG2 or multiple antigens SAG1 GRA1 ROP2 GRA4 SAG2C and SAG2X (Cong et al., 2005; Cong et al., 2014). A wider range of protective antigens would be generated by the second candidate vaccine because it contains epitopes which exist in both tachyzoite and bradyzoite forms of *T. gondii (*Mohammadhasani et al., 2024*)*.

3.3. Using an Adjuvant to Increase Immunity

The Adjuvant System (AS) presents favorable potential for use in *T. gondii* vaccine through its combination of aluminum salts primarily with oil-water emulsions and liposomes and immuno-stimulators. The combination of AS compounds including aluminium salts and oil-water emulsions and immune-stimulators could fit the requirements for cellular *T. gondii* protection through Th1-bias responses. MPL functions as the main component in all three systems because it represents a chemically modified form of Salmonella Minnesota R595 lipopolysaccharide and activates toll-like receptor 4 (Parmaksız et al., 2022). The stimulation of TLR4 through MPL treatment results in maturation of antigen presenting cells that subsequently increase cytokine production of IFN-ɤ and IL-2 and leads to Th1 immune response development (Gustafson, 1992). Various delivery vehicles serve as adjuvants when implementing *T. gondii* recombinant protein vaccines in experimental studies. Poly (lactide-co-glycolide) (PLG) biodegradable and biocompatible polymers have received substantial interest for *T. gondii* vaccine development due to their increased immunogenic characteristics. PLG NPs offer sustained in vitro antigen release functionality along with cellular uptake benefits for antigen presenting cells (APCs) and protect antigens from the degradation process occurring in vivo (Danhier et al., 2012). Cellular and humoral immune responses received double enhancement alongside extended survival duration from using natural chitosan nanosphere polymers with *T. gondii* lysate antigens (El Temsahy et al., 2016).

3.4. Effective Presentation and Distribution of Toxoplasma Antigens

The successful delivery of cell interior antigens plays an essential role together with membrane antigens to improve their recognition and presentation by host cells. Different types of live antigen delivery systems utilize *S. Typhimurium* bacterial vector alongside Bacille Calmette–Guerin (BCG) vector along with adenoviral vector and baculovirus vector for their employment. When vaccine is delivered the pre-existing vector immunity generates strong B and T memory cell responses against the vector which might lead to early pathogen clearance and decreased immunogenicity along with reduced gene expression duration (Chirmule et al., 1999). Researchers have proposed chimpanzee-specific adenoviruses as an alternative vector to transfer *T. gondii* antigens to humans since these adenoviruses show minimal pre-existing immunity in human patients (Dicks et al., 2012). This paradigm in parasite therapy has only just begun its development process. Scientists have not yet identified any reported probiotic microorganisms that act as *T. gondii* antigen carriers. It is reported that gut commensal microflora stimulates dendritic cells indirectly for protection against *T. gondii* infection (Benson et al., 2009). Most of the probiotic strains among lactic acid bacteria maintain high levels of resistance. Certain strains endure gastric conditions to colonize gastrointestinal tissue where they stay longer to display antigens to the immune response. *T. gondii* vaccine carrier has valuable potential because these probiotic strains show both tissue-colonization talents and the ability to stimulate DCs (Brito et al., 2023).

3.5. Effective Immunization Strategies

Heterologous prime-boost vaccination combines distinct vaccinations for the same pathogen to enhance protection against the target pathogen. Mendes et al. validated the effectiveness of dual adenovirus and modified Vaccinia Virus Ankara vaccines that first used adenovirus vector AdSAG1 followed by modified Vaccinia Virus Ankara vector. Across two test groups the mice which received a heterologous vaccination series showed significantly reduced brain tissue cyst counts in comparison to mice that received only homologous vaccination using two doses of AdSAG1 (Mendes et al., 2013).

Numerous heterologous prime-boost vaccine methods have been tested but DNA prime-protein boost along with DNA prime recombinant viral boost have proven most effective in *T. gondii* protection (Lu et al., 2015). The combination of DNA vaccines and protein vaccines through this approach generated significant cellular immune responses together with memory B-cell activation for specific antigens while protein vaccines mainly targeted antibody production. The combination of DNA primer along with viral vector boost methods aimed to generate T-cell responses. The order of vector delivery in a prime boost schedule affects the generated immune response based on vector properties according to previous research findings (Kardani et al., 2016).

Conclusion

The main obstacle in creating a novel vaccine against toxoplasmosis is achieving both high safety requirements and sterile protection in a vaccine design. There is considerable worry about the reactogenicity of extremely immunogenic live attenuated strains. The majority of research has demonstrated that the level of protection produced varies according to the type of infection (chronic or acute, suggesting that there is insufficient cross-protection against distinct strains of *T. gondii*. Because *T. gondii* has a complicated life cycle, its antigenic composition or specificity might alter as it progresses through distinct developmental stages. As a result, immunization with stage-specific antigens can only provide protection during the specific stage. Through careful analysis and the use of techniques at every stage of vaccine development, the first human vaccination against *T. gondii* will be developed. Furthermore, increasing our understanding of toxoplasmosis immunity might help us create a vaccine that works.

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