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Recent Development and Intervention on the Parasitological Study of *Toxoplasma Gondii*

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Abstract

Toxoplasma gondii is a globally prevalent protozoan parasite and the causative agent of toxoplasmosis, a disease of medical and veterinary importance. This review provides a comprehensive review of recent developments in the parasitological study of *T. gondii*. This study highlights advances in epidemiological surveillance, molecular diagnostics, and intervention strategies. The genetic diversity and virulence of *T. gondii* strains have been clarified through modern genotyping techniques such as restriction fragment length polymorphism (RFLP) and whole-genome sequencing, leading to improved classification and understanding of transmission dynamics. The application of genome-editing technologies, particularly CRISPR/Cas9, has enabled precise identification of genes critical for parasite survival and pathogenicity, particularly in response to host-derived oxidative stress. The emergence of organoid and three-dimensional (3D) culture models has bridged the gap between traditional *in vitro* and *in vivo* studies, providing physiologically relevant systems to investigate host-parasite interactions. Despite these advances, challenges remain in global genotyping, therapeutic development, and chronic infection management.

Keywords: Toxoplasma gondii; toxoplasmosis; epidemiological surveillance; genotyping; CRISPR/Cas9; molecular diagnostics; organoid models; 3D culture; oxidative stress; microbiome; intervention strategies; parasite virulence.

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1. Introduction

Toxoplasma gondii is a prevalent protozoan parasite that infects nearly one-third of the global human population, with seroprevalence rates varying widely by region due to climate, age, immune status, geography, dietary habits, and social customs[1-3].It is the causative agent of toxoplasmosis, a disease of medical and veterinary concern. The parasite's complex life cycle involves felids (Cats) as definitive hosts, where sexual reproduction occurs, and a wide range of warm-blooded intermediate hosts, including humans[4, 5] .Transmission to humans primarily occurs through ingestion of tissue cysts in undercooked meat, oocyst-contaminated water or soil, or through congenital transmission from mother to fetuses[2, 5, 6] .Although less common, *T. gondii* can also be transmitted through organ transplantation or blood transfusions from infected donors[4, 7].While often asymptomatic in immunocompetent individuals, *T. gondii* causes severe complications in immunocompromised patients, such as those with HIV/AIDS or organ transplants, and during pregnancy, leading to congenital infections associated with miscarriage, stillbirth, or long-term neurological disorder [7-9].

Diagnosis remains a challenge due to the intracellular nature of parasites and the limitations of conventional serological tests, which detect parasite-specific antibodies such as IgG and IgM in patient serum using enzyme-linked immunosorbent assay (ELISA) and immunofluorescence antibody assay (IFA) methods. Currently, there are limitations and ineffective treatments for toxoplasmosis, with no ideal treatment for the chronic stage of the infection due to toxicity and poor efficacy[10, 11].

Over the last two decades, significant developments have emerged in the parasitological study of *T. gondii*, ranging from advanced molecular diagnostics to innovative therapeutic and preventive strategies. This review examines recent breakthroughs and interventions that have transformed the understanding, diagnosis, and management of toxoplasmosis. Toxoplasma gondii, the causative agent of toxoplasmosis, has been the focus of extensive parasitological research due to its global impact on human and animal health.

Hence, recent studies have highlighted advancements in diagnostic techniques, genetic profiling, and intervention strategies.

2. Parasitological Studies and Advances in Diagnostic Techniques on Toxoplasma Gondii

2.1. Epidemiological Surveillance and Genotyping

In the early 1990s, *T. gondii* was considered genetically clonal compared to other protozoan pathogens.[5] Initial studies, such as the 1992 examination of single-nucleotide polymorphisms (SNPs) in the SAG1 and ROP1 loci using restriction fragment length polymorphism (RFLP) in *T. gondii*, led to the classification of *T. gondii* into two clones with different virulence in mice[12] .Subsequent RFLP analysis of six loci in 106 strains isolated from Europe and North America resulted in reclassification into three clones: types I, II, and III. This three-clone classification model has been confirmed by analyses using microsatellite markers, gene open reading frames, intron sequences, and RFLP research[13] .The "3-clone hypothesis" corresponded to the results of analyses of strains isolated in Europe and North America and became widely accepted.

However, more recent studies have identified clones distinct from types I – III, particularly in South America and among wild animals in North America[14, 15].Many atypical clones with RFLP patterns that do not correspond to the established types I-III have been noted, but recent RFLP analysis has shown that their mutations result from frequent recombination among them[16-19] .Types I-III differ in their virulence in mammals[14, 15] .Type I is highly virulent and exhibits a 100% lethal dose (LD100) with a single parasite-injected mouse. Type II exhibits low virulence with a 50% lethal dose (LD50 >103), and type III is a virulent (LD50 >105) and predominantly isolated from livestock. Quantitative trait locus (QTL) analysis, through crosses between highly and less virulent clones, has identified major virulence factors such as ROP18 by crosses between types I and II and types I and III[15, 19, 20] .This targets the host endoplasmic reticulum-bound transcription factor ATF6β and phosphorylates immunity-related GTPases (IRGs) to evade host clearance[21-23].ROP5, although lacking intrinsic kinase activity, collaborates with ROP18 in this process to phosphorylate IRGs, and is present in high copy number as a tandem repeat in the *T. gondii* genome[23-25]. QTL analysis also revealed ROP16, which modulates host immune response via STAT3 and STAT6 phosphorylation, with leucine 503 being critical for STAT3[19, 20, 24, 26].

Advances in genome-wide SNP analysis of 62 strains have led to the reclassification of *T. gondii* into six clades and sixteen haplogroups[16, 27] .However, these studies primarily utilized isolates from Europe, North and South America, and Africa, with limited representation of isolates from Asia and Oceania to allow characterization. Consequently, further studies are necessary to achieve global genotyping of *T. gondii*, particularly in Asia, where there are few molecular epidemiological studies and limited information on haplogroups.

Genotyping studies have revealed the genetic diversity of *T. gondii* strains, which vary in virulence and geographical distribution. Advances in whole-genome sequencing have facilitated detailed population studies, improving our understanding of transmission patterns and outbreak sources. Studies in South America, for instance, have highlighted the emergence of atypical, highly virulent strains. Studies emphasize the importance of genotypic characterization to understand the parasite’s pathogenicity and virulence. Genetic profiling helps identify strains with increased resistance to drugs and vaccines.

2.2. Emerging Research Frontiers

A. CRISPR/Cas9 Genome Editing

Recent advances in genome editing, particularly in the application of Genome-wide CRISPR/Cas9 screening, are powerful tools for identifying essential genes under a specific condition and studying the molecular mechanisms associated with specific phenotypes. Currently, screening with genome-wide CRISPR/Cas9 gene-editing technology has been successfully applied to human cells and microorganisms [28, 29] and has been used to identify previously uncharacterized functional genes in *T. gondii*[30, 31] .The dense granule protein GRA45 in *T. gondii* is a virulence factor identified using genome-wide screening[32] .Hence, the genome-wide CRISPR/Cas9 enables precise, targeted gene knockouts, thereby facilitating the identification and characterization of virulence factors and other gene critical to parasite survival and pathogenicity.

According to Chen, and his colleagues [33], a genome-wide CRISPR/Cas9 loss-of-function screening was performed on the *T. gondii* RH strain gene in order to identify potential genes contributing to the ROS stress response. On treatment with hydrogen peroxide, 30 single guide RNAs targeting high-confidence genes were identified, with the inclusion of some known important antioxidant genes such as catalase and peroxiredoxin PRX3. Also, previously uncharacterized genes were identified, among which five hypothetical protein-coding genes, such as HP1–HP5, were selected for further functional characterization. The deletion of the HP1 in *T. gondii* RH led to significant sensitivity to H2O2, suggesting that HP1 is vital for regulating oxidative stress. In addition, with the loss of the HP1 gene, which led to the decreased in antioxidant capacity, invasion efficiency, and proliferation in vitro. In vivo, this also revealed that the survival time of mice infected with the HP1-KO strain was significantly prolonged relative to that of mice infected with the wild-type strain. Altogether, these findings demonstrate that the CRISPR/Cas9 system can be used to identify potential genes critical for oxidative stress management, and the HP1 gene may offer protection against oxidative damage and contribute to *T. gondii* virulence in mice.

B. Organoid and 3D Culture Models

Traditional investigations on *T. gondii* parasitism have been divided between in vitro, such as 2D cell cultures, in which mammalian cells are grown on flat surfaces, and animal infection models[34] .Infections of monolayers (2D) of mammalian cells plated in culture dishes represent a cheap and convenient system for viewing infected cells and performing controlled studies with limited variables[35]. However, the structural constraints of growing cells on flat surfaces in culture dishes yield cells with artificial shapes and different architecture than in animal tissues; thus, it does not recapitulate natural infections. Animal models are used to investigate cyst burden and distribution in tissues, immune responses, and pathogenicity of chronic infections[34] .

According to Tedford and McConkey [34], rodents are commonly used to study infections as these animal models reproduce the neurological symptoms associated with toxoplasmosis. Genetically bred mice with altered immune systems have been developed to parse out immune pathways and responses. The development of a bioluminescence system in T. gondii (luciferin/luciferase), in addition to other genetic modifications of the parasite, has enabled monitoring of the spread of an active infection in living animals [6]. However, animal models of individual infections provide little experimental precision and do not permit examination of parasite-host cell interactions at the subcellular level; they can also be time- and money-intensive. Recent advances in stem cell biology and tissue engineering have led to the development of organoid and three-dimensional (3D) culture models, which are transforming the study of host-pathogen interactions. This bridges the 2D monolayer and whole animal methods. Culturing a 3D matrix range of cell types can mimic the morphological and functional features of cells and tissues in vivo and provides a physiologically relevant model system to investigate host-parasite interactions. The altered morphology of cells grown in 2D cultures as flat monolayers may likely impact the parasite and PV morphology, as a result of the mechanical forces acting on the infected cell and the pressure of the culture medium.

A notable study by Seo, and his colleagues [36], uses human cerebral organoids to model *T. gondii* infection. These brain organoids, generated from human pluripotent stem cells, can recapitulate the pathophysiology of in vivo human brain tissue, constituting a valuable resource for studying neurotropic pathogens. In a recent research, a new in vitro model was developed, this demonstrate that *T. gondii* tachyzoites are capable of infecting the human brain organoids, where there transformed into bradyzoites and replicate within parasitophorous vacuoles to form cysts, this indicates that the asexual life cycle of *T. gondii* can be efficiently simulated in the brain organoids, providing a unique opportunity to observe parasite development and the persistence in a host-parasite relevant[36]. However, transcriptomic analysis of infected organoids of *T. gondii* revealed the activation of the type I interferon immune response, indicating the organoid's ability to mount innate immune defence similar to that seen in vivo. The infections also induce a change in the *T. gondii* transcriptome, particularly in genes related to protozoan invasion and replication.

C. Microbiome-Interaction Studies

According to Yang, and his colleagues [37], the gut microbiome is increasingly recognized as a key player in shaping the outcome of *Toxoplasma gondii* infection. The primary route of *T. gondii* infection in mammals is usually through oral ingestion of contaminated water and undercooked food, leading to the release of bradyzoites in the intestinal epithelia; this is the vital step for establishing infection and proliferation of the parasite[37] .These bradyzoites, released from the cyst, invade the intestinal mucosa, differentiate into tachyzoites, which eventually disseminate to the nervous system and muscle tissue [38] .Early in infection, immune cells such as monocytes and neutrophils are recruited to the intestine, producing interleukin-12 (IL-12), and the secretion of interferon-gamma (IFN-γ) is activated to restrain the parasite growth[38] .However, the immune respond can cause significant like the overproduction of pro-inflammatory cytokines and nitric oxide, which can lead to severe pathological changes in intestinal tissue. Study reported that oral infection causes loss of Paneth cells, lowers the tolerance to antigens in the intestinal lumen, leakage of luminal contents into the submucosa to drive intestinal inflammation, and disruption of the epithelial barrier[37, 38] .The presence and composition of the gut microbiota profoundly trigger a more severe inflammatory response[37, 38].

The continuous secretion of factors relevant to *T. gondii* while invading the intestinal tissue results in drastic changes in the number and composition of gut microbiota[39] .An experimental study on mice orally infected with cysts of *T. gondii* in the intestinal microbiome reveals that ileitis is accompanied by increased bacterial load, decreased species diversity, and bacterial translocation, which is especially characterized by a significant reduction or disappearance of Bacteroidetes and Firmicutes[40]. Also, the loss of Paneth cells and absence of secreted antimicrobial substances significantly increases the number of Escherichia coli, which leads to accumulation of bacteria in the inflamed ileum, translocating to the lamina propria, and then spreading to other tissues[41] .Notably, germ-free animals treated with antibiotics to deplete gut bacteria exhibit reduced intestinal inflammation and significantly increase the survival rate following *T. gondii* infection, highlighting that the gut microbiota can affect the process of toxoplasmosis[40] .The gut microbiota coevolves with the intestinal immune system of the host, which plays an important role in the expression of regulatory immune mediators and in the development, recruitment, and differentiation of immune cells[39] .Studies have demonstrated that the combined utilization and recolonization of Escherichia coli, Prevotella, and Lactobacillus johnsonii in mice are effective in restoring susceptibility to toxoplasmosis and modulating immune responses by producing NO and IFN-γ in the intestine[40]. These findings suggest that gut microbiota regulation can be used to remedy parasitosis. Probiotic treatment or fecal microbiota transplantation (FMT) in the host is certified to be beneficial during *T. gondii* infection[42]. Recent research has also explored the interplay between the microbiota and *Toxoplasma* infection[38, 41, 42] .Utilization of 16S rRNA gene sequencing and Metabolomic analysis identified alpha-linolenic acid (ALA), a gut metabolite, using Spearman correlation analysis, positively correlated with the beneficial bacterial genera and reduced inflammation[43, 44]. Oral administration of ALA in mouse models of *T. gondii*-induced colitis alleviated intestinal inflammation and improved the dysregulation of gut microbiota, suggesting potential therapeutic application[45]. Furthermore, *T. gondii*α-amylase (α-AMY), a key enzyme in amylopectin metabolism, plays an essential role in maintaining chronic infection and may interact with gut microbiota[46] .Knocking out of α-AMY in *T. gondii* significantly affects the cyst; this results in a decrease in the severity of toxoplasmosis, which is increasingly attributed to the gut microbiota[38]. These further support the theory and previous study results of mouse models infected with cysts of different virulence, which were established to explore how gut microbiota affects *T. gondii*-induced intestinal inflammation[42].

2.3. Molecular Diagnostics

Molecular diagnostic approaches have subsequently evolved, offering improvements in both sensitivity and specificity. Among these, nucleic acid amplification tests (NAATs) such as polymerase chain reaction (PCR), nested PCR, real-time PCR, loop-mediated isothermal amplification (LAMP), multiplex PCR, and PCR–restriction fragment length polymorphism (PCR–RFLP) are increasingly used.[47] These methods complement conventional serological assays for diagnosing *T. gondii* infection, which include the Dye Test (DT), Agglutination Test (AT), Modified Agglutination Test (MAT), Latex Agglutination Test (LAT), Enzyme-Linked Immunosorbent Assay (ELISA), and Western BlotPCR, a widely utilized molecular diagnostic method, involves the amplification of genes specific to *T. gondii*[18]. It enables the detection of infections at very low concentrations and quantifies tachyzoites of T. gondii in tissue samples that cannot be measured by traditional serological methods[47] .

Recent research has indicated the development of an alternative genetic target to address the limitation of the B1 gene[15, 40] .The p30 gene, which offers greater sensitivity than the B1 gene, studies indicate that it has lower specificity when compared to the B1 gene, and cross-amplifies with DNA from Nocardia and Mycobacterium tuberculosis[48]. Furthermore, the rep529gene, a gene fragment that is repeated 200–300 times in the *T. gondii* genome, can detect 60 strains among *T. gondii* strains[49] .This demonstrates the superior sensitivity of the rep529 gene compared to that of the B1 and p30 gene, enabling the detection of a broad range of positive samples missed by some commercialized PCR-based diagnostic kits[50] .Consequently, the B1 gene and the therp529 gene are commonly targeted due to the advantages they may offer. However, studies focusing on genes such as SAG2, GRA7, ROP8, and ITS-1 to further enhance the diagnostic sensitivity but also specificity[5, 14, 15, 32, 51, 52].

Advancements in conventional PCR, such as the nested-PCR, real-time PCR, real-time PCR–High-Resolution Melting Mechanism (PCR–HRM), loop mediated isothermal amplification (LAMP), multiplex PCR, and PCR–restriction fragment length polymorphism (PCR–RFLP), have increased the sensitivity of various target genes, this have further advanced diagnosis[5, 14, 15, 32, 51, 52]. Real-time PCR coupled with High-Resolution Melting Mechanism (PCR–HRM), a method of analyzing DNA characteristics of several genes and differentiating the genotype of *T. gondii* infection through a simple and cost-effective means[18, 51, 52].

A significant invention of Digital Droplet PCR (ddPCR) has been developed to improve and enhance the use of PCR[53].This technique utilizes the amplification of single DNA templates to directly quantify parasitic infections, thereby increasing its sensitivity and specificity for detecting of very low-level parasite strain[53] .This process produces a linear digital signal of the DNA product, which enables the device to detect mutations[53]. These technologies allow for the precise detection, quantification of low parasite load and to differentiate between related parasite species, In one study, ddPCR demonstrated high sensitivity and specificity compared to real-time PCR, achieving 100% sensitivity and specificity in 80 mussel DNA samples, making ddPCR a more preferable than conventional PCR in diagnosis and screening and also addressing the quantification in accuracy often encountered with qPCR[53, 54].

2.4. Serological Improvements

Enzyme-linked immunosorbent assay (ELISA) is one of the most widely used serological diagnostic methods for *T. gondii* due to its high sensitivity and specificity[47]. Different ELISA types have been developed, using *T. gondii* tachyzoite lysate antigen, chimera or recombinant protein antigen, or *T. gondii*-specific antibodies to facilitate the detection of antibodies or their antigen in samples such as serum or plasma[8, 10]. Primarily, the diagnosis is based on the detection of *T. gondii*-specific IgG or IgM in laboratory samples[49]. The production of this IgG and IgM antibody varies with time after infection with *T. gondii*, which enables the classification of the infection into acute and chronic infections based on the measurements of IgG and IgM[54, 55] .IgM is typically detected in acute infections, but it can persist for up to a year. The diagnosis of both acute and chronic infections is made by measuring IgG levels simultaneously rather than relying on IgM detection alone[54, 55]. In order to improve diagnostic sensitivity, the IgM assay is often followed by an IgG antibody avidity test, which provides an estimate of the time of infection[4, 6, 55] .Commercial ELISA kits are useful not only for clinical diagnosis in humans but also for preventive screening in animals such as sheep and pigs [4, 6, 55]. Indirect ELISA involves coating the bottom of an immune plate with *T. gondii* antigens and incubating it with serum antibodies[2, 48]. Most ELISA assays use *T. gondii* tachyzoite lysate antigen (TLA) for the detection of IgG or IgM antibodies, and show substantial agreement with other serological methods such as MAT, IHA, or IFAT[53, 56] .

However, ELISA using TLA has its limitations, including potential contamination by impure substances and the possibility of false negatives or false positives due to the purification processes of parasites cultured from living mice and cells, as well as cross-reactivity with antigens of other closely related parasites or between proteins of *T. gondii*[57, 58]. Despite these drawbacks, ELISA remains the most widely used diagnostic method because it allows for the simultaneous testing of a large number of samples and the detection of both IgG and IgM antibodies.

Recent advancement has focused on the development of ELISA assays using recombinant antigens, which have demonstrated high sensitivity and specificity in the detection of *T. gondii*-specific IgG or IgM antibodies (Huertas and his colleagues, 2021[58-60] .These assays also offer the advantage of differentiating between acute and chronic toxoplasmosis. Recombinant antigens studied include surface antigens involved in host cell invasion, such as SAG1, SAG2, and SAG3, as well as dense granule antigens (GRAs) like GRA1, GRA4, and GRA7, which are important for parasite persistence[5, 15, 57, 61, 62] .

To overcome the limitations of conventional ELISA, recent studies have investigated the use of nano-gold particles synthesized with antigens or antibodies coated on plates. In one study, they developed a nano-gold ELISA by synthesizing SAG1 pAb with gold nano-particles (AuNPs) demonstrated higher sensitivity, specificity, and diagnostics accuracy compare to compared its performance with that of conventional ELISA[63, 64].The nano-gold ELISA showed a sensitivity of 89.2%, higher than the 83.8% observed with the conventional ELISA. In addition, the specificity and diagnostic accuracy were 94% and 91.95%, respectively, also exceeding the conventional ELISA values of 88% and 86.2%[65, 66] .Similarly, a nano-ELISA developed with E/S antigen and AuNPs achieved a significantly higher sensitivity and specificity of 93.33% than 80% sensitivity and 86.66% specificity of the traditional ELISA[67] .The improved performance is attributed to the increased surface area provided by the nanoparticles, which facilitates a greater number of antigen–antibody interactions[47, 49].

3. Intervention and Therapeutic Advances on Toxoplasma Gondii

3.1. Drug Development

Current treatment strategies for toxoplasmosis primarily rely on a pyrimethamine-based regimen, comprising pyrimethamine, sulfadiazine, and folinic acid (leucovorin) [68]. Pyrimethamine act as a folic acid antagonist by inhibiting Dihydrofolate Reductase (DHFR) enzyme, thereby blocking the synthesis of purines and pyrimidines which are precursors for synthesis of DNA and cell multiplication[65, 69, 70] .Sulfadiazine, a sulfonamides, further disrupts folic acid synthesis by competitively inhibiting the dihydropteroate synthetase (DHPS) enzyme. Folinic acid is co-administered to mitigate the risk of myelosuppression, associated with pyrimethamine[71]. Despite its effectiveness, this combination is contraindicated during the first trimester of pregnancy due to teratogenic risk. Although rare, it can cause several adverse effects, such as agranulocytosis, Stevens-Johnson syndrome, toxic epidermal necrolysis and hepatic necrosis[70, 71]. Alternative regiment such as pyrimethamine combined with clindamycin, clarithromycin, azithromycin or atovaquone, as well as monotherapy like cotrimoxazole (trimethoprim-sulfamethoxazole) or atovaquone has been explored, but none have demonstrated superior efficacy over conventional treatment[65, 68, 69].

Although standard chemotherapy has been shown to reduce the risk of toxoplasmosis-related sequels and symptoms associated with congenital infection, when administered promptly, it does not eliminate tissue cyst, which remain dormant and reactivate if the host's immune system weakens[65, 68, 70, 72, 73]. Spiramycin, a macrolide antibiotic, is used as a preventive therapy during suspected maternal infection due to its accumulation in the placenta, thereby reducing transplacental transmission of *T. gondii*. However, if fetal or neonatal toxoplasmosis is confirmed, Spiramycin is discontinued and replaced by conventional therapy[71]. Adjunctive corticosteroids, such as dexamethasone, are sometimes used in ocular toxoplasmosis to manage inflammation with combination with antimicrobial therapy. With ongoing phase II clinical trials evaluating the optimal dosage treating brain edema in an HIV-infected patients exhibiting cerebral toxoplasmosis [37]. The limitation of current therapy, such as the harsh profile of side effects and treatment duration (from 4–6 weeks to over 1 year), which may affect compliance[74]. Increasing drug resistance, different drug susceptibility for different pathogenic strains and the remaining unknown aspects of the pathogenicity of *T. gondii* infection, which plays a vital role in drug failure and disease progression[75]. Also, the lack of efficacy against tissue cysts from the infected host, this underscore the urgent need for novel, safer, and more effective treatment options[74].Presently, immunization remedies are currently being developed, but there seems to be no vaccine available for human administration.

3.2. Vaccination Strategies

Efforts to develop effective vaccines against Toxoplasma gondii are ongoing, particularly for veterinary use in livestock to prevent transmission to humans. An ideal human vaccine should not only reduce infection and mortality rates but also lessen the burden of chronic cases that require long-term care, while remaining economically viable[32] .Immunity to *T. gondii* is known to increase following infection, with both humoral and cellular immune responses playing significant roles in protection. Over the past 30 years, significant efforts have been made to develop vaccines against toxoplasmosis using a variety of strategies, including killed and attenuated organisms, tachyzoite-lysate antigen, excretory-secretory (ES) antigens, somatic proteins, DNA vaccines, and recombinant proteins[62].DNA vaccines, recombinant antigen-based vaccines, and nanoparticle-delivered formulations have shown protective effects in animal models, though no human vaccine is yet available[76] .The development of live-attenuated vaccines for use in sheep and goats has made considerable progress in reducing vertical transmission. However, none of these approaches has produced consistently effective immunity in tested antigens[77, 78].Despite these extensive efforts, only the commercial vaccine TOXOVAX has reached the market[62].TOXOVAX is a subunit vaccine containing live attenuated tachyzoites from the RH strain (48S), which have lost the ability to form tissue cysts in the host[62, 77].This vaccine is used exclusively in veterinary medicine, with limitations such as its unsuitability for use in humans, especially during pregnancy, and its short-term protective effect. Vaccination with the RH strain in mice has been unsuccessful, although some other strains have shown limited success when combined with immune stimulation[61].

Ionizing radiation, including gamma rays, has long been considered in vaccine and drug development. This technology is used for the development of human and animal vaccines, sterilization, and the induction of random mutations[11].It has been demonstrated that gamma rays can destroy the infectivity of *T. gondii* cysts, and other electromagnetic rays, such as ultraviolet, can inhibit tachyzoite proliferation and tissue cyst formation[32].By exposing *T. gondii* tachyzoites to controlled doses of gamma radiation, their pathogenicity can be weakened while preserving their antigenic properties, making gamma radiation-attenuated tachyzoites a promising vaccine candidate[76].

A recent study by Pourmohammadi, and his colleagues [76] investigated the potential of gamma radiation-attenuated tachyzoites of the RH strain as a vaccine. In this study, tachyzoites were exposed to gamma rays at doses of 50, 100, and 200 Gy. Viability was assessed using flow cytometry, and infectivity was evaluated in HeLa cell cultures. An in vivo study was conducted by injecting irradiated tachyzoites into BALB/c mice, with disease progression compared to a control group challenged with intact tachyzoites. Serum levels of interleukins 2 and 10, and interferon-gamma were measured before and after the vaccine challenge. The results showed a significant, dose-dependent mortality rates in tachyzoites exposed to gamma rays, with 35.28%, 58.31%, and 89.28% mortality at 50, 100, and 200 Gy, respectively. In HeLa cell cultures, the gamma ray exposure significantly reduced the tachyzoite load when compared to the control group. In vivo, mice infected with 50 Gy-irradiated tachyzoites showed complete mortality within 14 days, while the 100 Gy group had partial mortality (4 out of 20 died), and the 200 Gy group had full survival. Control mice infected with intact tachyzoites died within 8 days (average survival: 6.8 ± 0.44 days). ELISA results indicated significant increases in cytokine levels (IL-2, IL-10, IFN-γ) in mice treated with 100 Gy and 200 Gy irradiated tachyzoites after the vaccine challenge[29].Survival monitoring revealed higher survival rates in the 100 Gy and 200 Gy groups compared to controls. From this study it shows that gamma radiation-attenuated tachyzoites represent a promising avenue for vaccine development, providing insight into the potential use of gamma radiation to reduce the pathogenicity of *T. gondii* and enhance the immune response in infected hosts[76] .Also further studies are needed to ascertain their safety, particularly in human populations.

4. Conclusion

The past decade has seen remarkable progress in *Toxoplasma gondii* parasite research, including advanced molecular diagnostics, innovative therapeutic approaches, and promising vaccine research. High-throughput techniques such as genome-wide CRISPR and Cas9 have revealed new genetic determinants of parasite virulence and stress resistance, opening avenues for targeted interventions. Despite these improvements, toxoplasmosis remains a major global health challenge, particularly in resource-limited settings, due to the lack of effective long-term treatments, the lack of a human vaccine, and the complexity of controlling zoonotic transmission. Only through an integrated effort that bridges the gap between laboratory discoveries and real-world prevention will we be able to eradicate the scourge of piracy.

5. List of Abbreviations

2D	Two-Dimensional
3D	Three-Dimensional
ATF6β	Activating Transcription Factor 6 Beta
CAT	Catalase
CRISPR/Cas9	Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9
ELISA	Enzyme-Linked Immunosorbent Assay
H2O2	Hydrogen Peroxide
HIV/AIDS	Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome
IFA	Immunofluorescence Antibody Assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IRGs	Immunity Related GTPases
KO	Knockout
LD100	100% Lethal Dose
LD50	50% Lethal Dose
Prx	Peroxiredoxin
PV	Parasitophorous Vacuole
QTL	Quantitative Trait Locus
RFLP	Restriction Fragment Length Polymorphism

ROP1	Rhoptry Protein 1
ROS	Reactive Oxygen Species
SAG1	Surface Antigen 1
SNP	Single Nucleotide Polymorphism
SOD	Superoxide Dismutase
STAT3/6	Signal Transducer and Activator of Transcription 3/6
T. gondii	Toxoplasma gondii
TR	Thioredoxin Reductase
Trx	Thioredoxin

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