RCMOS - Multidisciplinary Scientific Journal The Knowledge.
ISSN: 2675-9128. São Paulo-SP.

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

Effectiveness Of Vaccines Under Study for Toxoplasmosis: A Literature Review Of The Years 2016 to 2021

Giovanna Fausto Signori
João Vitor Senedese Leite Ribeiro

Abstract

Introduction: Toxoplasmosis is a disease with high prevalence worldwide, being related to public health problems. This disease is transmitted horizontally, with the ingestion of bradyzoites or oocysts, and vertically, which occurs via the placenta, with both routes of transmission being present the sexual and the asexual reproduction, depending on which animal is being contaminated. These are related to the acute phase, with hemato lymphatic dissemination and the production of symptoms, and the chronic phase of the disease, which is directly associated with the formation of cysts with bradyzoites inside the host's cells, with a reduction of symptoms. This scenario, in conjunction with the reality of the high cost of drugs to treat the condition and the range of symptoms that overcrowd the local health system, demonstrates the need for the development of vaccines, which act preventively, and there are several studies in progress. **Methods/Development:** a search was conducted in the SciELO, PubMed and Lilacs platforms with the words Active immunotherapy, toxoplasmosis, humoral immunity and cellular immunity, and 32 articles were found. Inclusion criteria were thus established as delimitation between the years 2012 and 2021, articles available in Portuguese, English and Spanish, studies of new vaccines for toxoplasmosis that were performed in mice. In addition, an exclusion criterion was established for in vitro studies, and thus 18 articles were selected, from which the data for this study were extracted. **Objectives:** to synthesize the results obtained with the vaccines under study and to understand where the process of their development stands. **Results/Discussion:** DNA vaccines were identified with the study, which are easy to produce and low cost, and have all been associated with the generation of humoral and cellular response and thus immunization ability; they have been present in several studies, being composed of the high virulence Toxoplasma gondii that have dense granule proteins (GRA), surface antigens (SAG), rhoptry proteins (ROP), microneme proteins (MIC) and plant-like calcium-dependent protein kinases (CDPKs). **Conclusion:** thus, DNA vaccines are superior, due to the ease of production and low cost compared to the others. To evaluate the immunization capacity, it was identified that the vaccines with the highest humoral and/or cellular immune response were those composed of calcium-dependent protein kinases (CDPK3), MIC3/ROP9/SAG2 junction and mitochondrial rhomboid proteases (ROM4) added to peptide.
Finally, it was found that the vaccine with SAG5B/SAG5C surface antigens showed the highest capacity to reduce parasite load, being the DNA vaccine that showed the highest protective effectiveness against toxoplasmosis.
Keywords: Toxoplasma gondii. Vaccinate. Immune Response. Protection.

INTRODUCTION

Parasitoses in Brazil have a strong association with socio-environmental factors such as basic sanitation and eating habits, for example (VISSER; GIATTI; CARVALHO; GUERREIRO, 2011). Toxoplasmosis is one of them, being caused by the protozoan Toxoplasma gondii, which is an obligate intracellular parasite (NI et al., 2018). Despite having been discovered in Brazil in 1908, it is still a very prevalent disease in this and several other countries around the world, and the number of population contamination can reach more than 60% in its totality (NEVES et al, 2011).

In man, the transmission of Toxoplasmosis can be done in three distinct ways: 1 - The oral fecal route is the most popular and most associated to this parasitosis, being infected cats the main

RCMOS - Multidisciplinary Scientific Journal The Knowledge.
ISSN: 2675-9128. São Paulo-SP.

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

vector of the disease. By defecating in places socially deficient in structures, such as basic sanitation, transmission occurs through the ingestion of oocysts, which are the infective forms of the parasite, in water and food that have been contaminated by humans; 2 - A little known way, but still prevalent and relevant in contamination, is the ingestion of meat from an infected animal, such as raw or undercooked pork or even lamb. That is, exotic cooking habits should be cautious in their preparation because there may be cysts in the muscle tissues of the animal, especially in endemic areas for this parasitosis; 3 - Finally, the transplacental route occupies a prominent place among the modes of transmission, because it becomes a congenital infection from the mother, contaminated and in the acute phase of the disease by trophozoites, to the newborn (LOVISON; RODRIGUES, 2017).

In both transmission mechanisms there are two phases: asexual and sexual. In the asexual reproduction phase, the free tachyzoite form stands out, which proliferates and disseminates through circulation or lymph, and may generate a polysymptomatic picture in the host. This is the acute phase of infection, and may cause a specific immune response or, in more severe cases, lead to the death of immunocompromised patients, abortions in contaminated pregnant women or serious pathologies if the pregnancy is concluded. In the chronic phase, there is the formation of cysts with the bradyzoite form inside, causing a reduction of parasitism and symptomatology of the individual, remaining this way for long periods, in the sporulated form (KAWAZOE, 2005) or even triggers severe symptoms in immunodeficient hosts, such as blindness and paralysis (HEALTH, 2009). The sexual phase, in turn, occurs only in the enteral cells of the jejunum, ileum and small intestine of cats that have contracted cysts (bradyzoites) or eggs of the parasite by the same ways as humans, ie, in food and meat from other contaminated animals and even in contaminated water. After the complete reproduction of the parasites in the cells, cats defecate millions of oocysts in the environment (sporulated form - very resistant to changes in the external environment), creating the possibility of the parasitosis spreading (BARBOSA; MUNO; MOURA, 2014). (FIGURE 1).

Toxoplasmosis has a variable clinical picture, manifesting itself in various ways, such as asymptomatic, acute febrile, generalized, affecting the lungs, myocardium, liver and brain; toxoplasmosis can also be ocular, which causes chorioretinitis in more than 40% of cases and can also cause acute and chronic retinitis; neonatal toxoplasmosis can cause microcephaly, hydrocephalus or other diseases. Thus, this parasitism can be asymptomatic or with various symptoms, reaching an extremely serious and lethal condition (LOVISON; RODRIGUES, 2017). From this information about the most severe forms, which manifest in neonates, it is understood the importance of developing prevention methods for pregnant women so that these cases have their rate of Complications decreased.

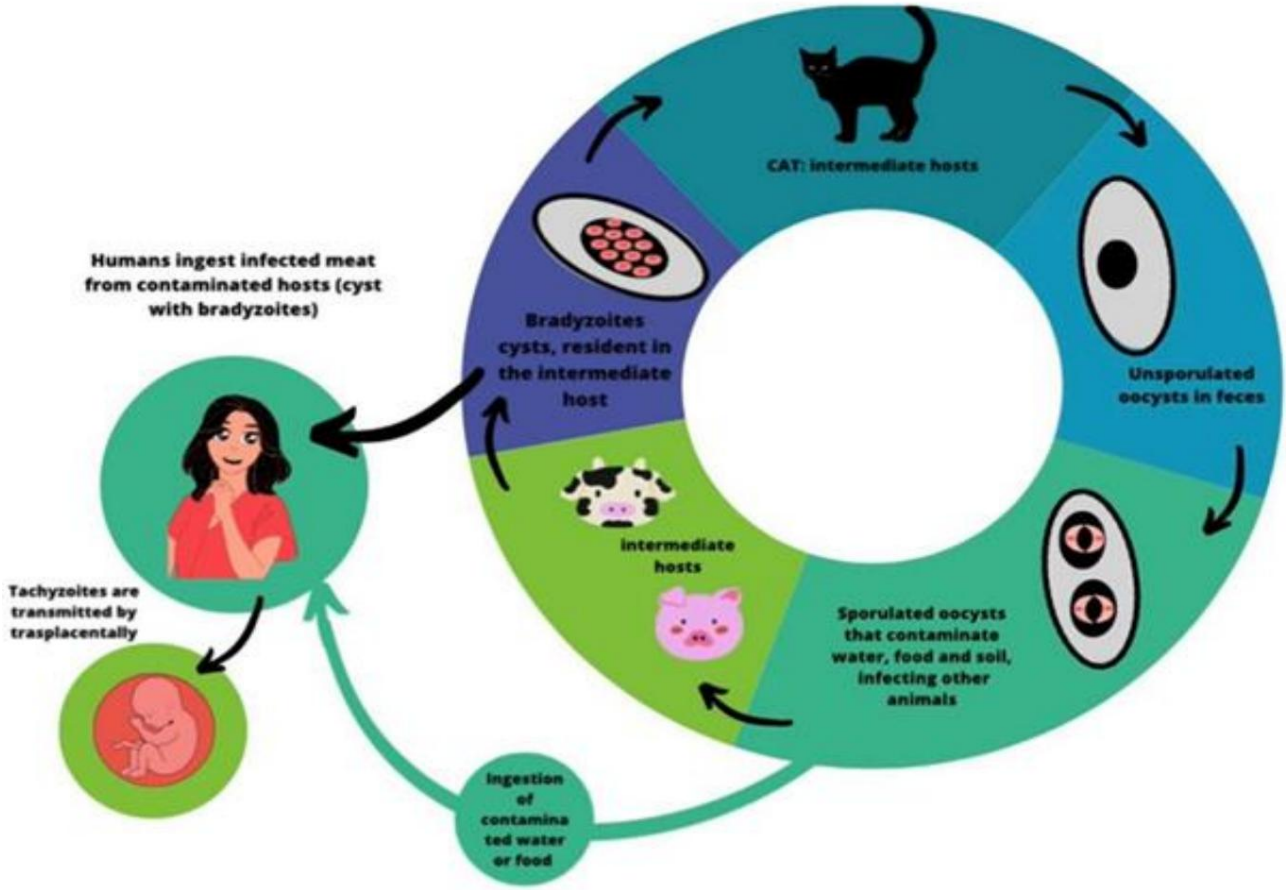
The toxoplasma gondii has shown to be a great risk for the world population, especially in regions with no guaranteed or even precarious basic living conditions. Furthermore, the drugs used in

RCMOS – Multidisciplinary Scientific Journal The Knowledge.
ISSN: 2675-9128. São Paulo-SP.

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

the treatment of this parasitosis, pyrimethamine and sulfadiazine, have suffered inflation of more than 1000% in their commercial value in recent years, which implies limited access to treatment, since it more often affects more vulnerable populations. It is important to emphasize that this factor also causes a burden on the health system directly and indirectly, that is, in the direct treatment of acute manifestations and subsequent treatment of chronic manifestations (CHU; QUAN, 2015). Thinking From this perspective, several researches have been done on the mechanisms of action, infection, and response to toxoplasmosis in humans in order to find a more efficient and inexpensive way to control symptoms and infection. From the perspective of host immune response to infection, vaccines began to be developed with different mechanisms of action and active ingredients such as synthetic peptides with multiple epitopes, recombinant adenovirus, DNA protein and recombinant eukaryotic plasmids, which showed positive results, which will be better described throughout this work (LANNES-VIEIRA, 2014). The vaccines under study so far, tested in mice, were able to trigger cellular and humoral immunity, as well as the reduction of cysts and the increase in survival time. From the analysis of this scenario, there is a need for the compilation of data from the vaccines under study, in order to understand where the process of their development stands. Therefore, this study aims to synthesize the results obtained with the vaccines under study.

Figure 1 - T. gondii cycle



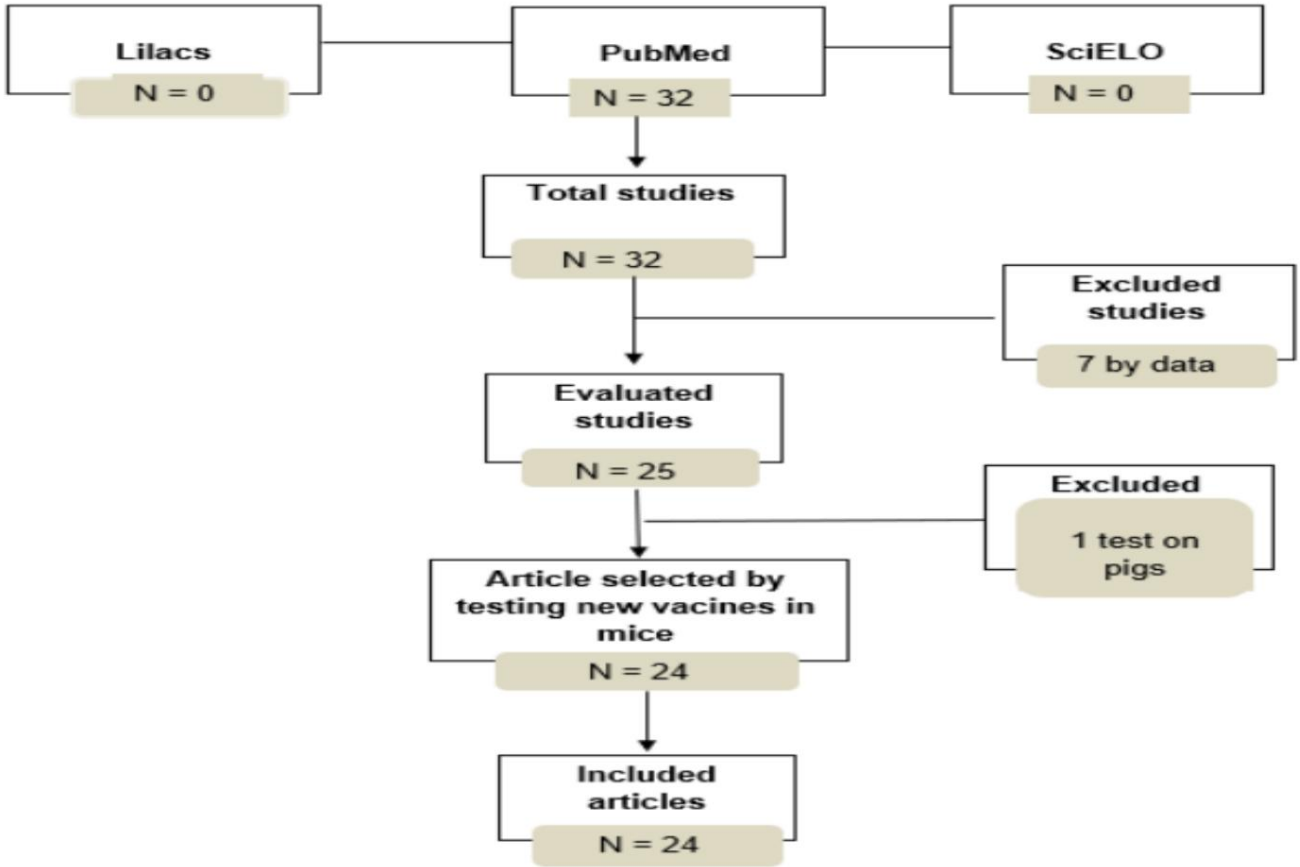
Source: own authority (2022).

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

METHODOLOGY

The SciELO, PubMed, and Lilacs platforms were searched using the descriptors Active Immunotherapy, Toxoplasmosis, Humoral Immunity, and Cellular Immunity, and 32 articles were found, all from PubMed. After that, a temporary and language delimitation was performed, including articles published between the years 2012 and 2021, available in Portuguese, English, and Spanish. Inclusion criteria were also established, such as studies that tested new vaccines, studies that tested on mice, and studied that DNA vaccines. The exclusion criteria were studies that performed in vitro tests, studies that used live attenuated vaccines, systematic review studies, and literature review studies. These criteria were based on the reduction of biases related to the different responses that in vitro and mouse studies can generate. For data extraction, the following questions were used: "Did the vaccine generate immunity?", "What is the vaccine constitution?", and "What is the mechanism of action of the vaccine?". With that the data about the vaccines were computed, so that the results could be identified.

Figure 2 – Methodology flowchart



DISCUSSION AND RESULTS

DNA Vaccines

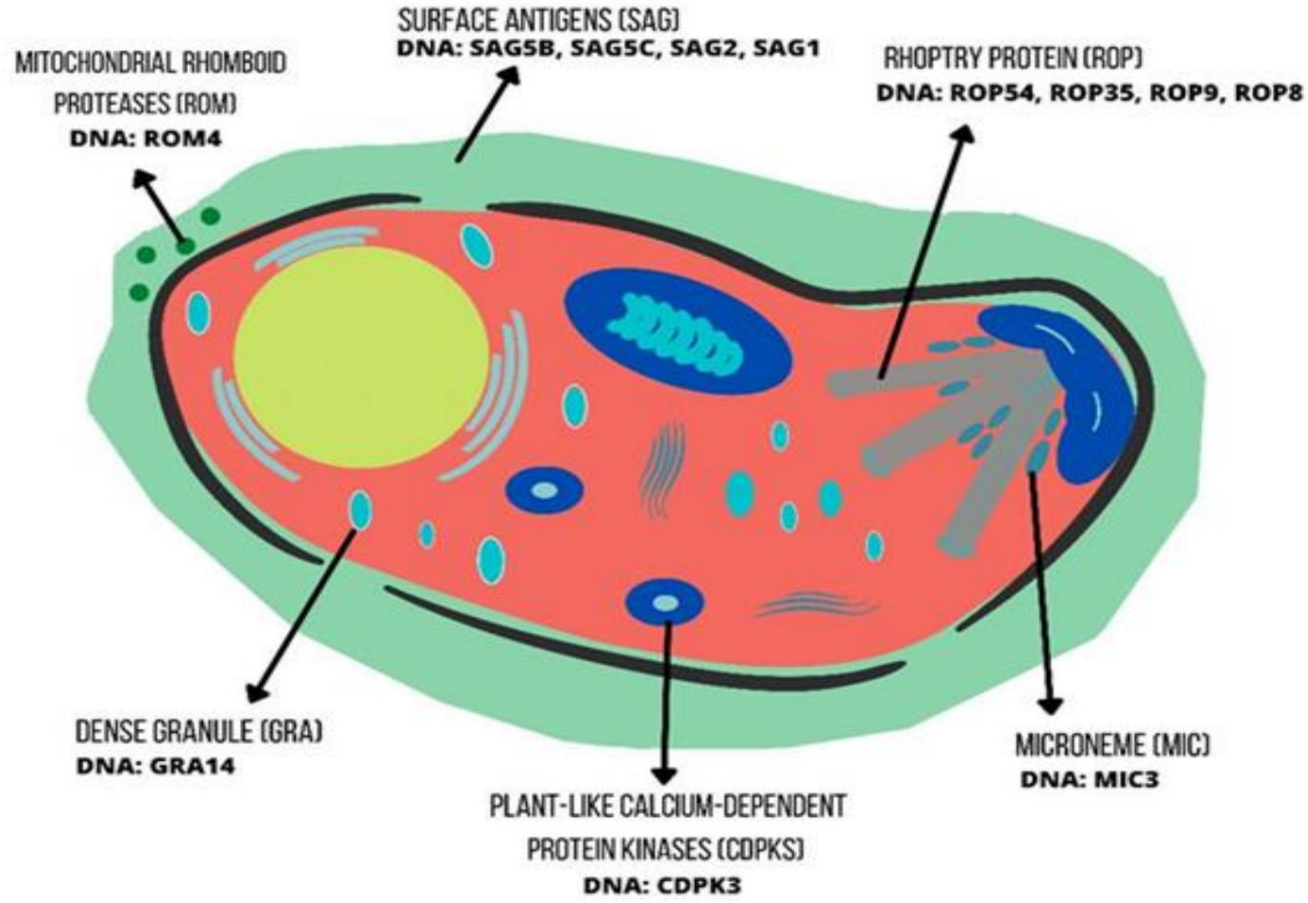
DNA vaccines, besides being the most efficient so far, are the easiest to produce, have low

RCMOS – Multidisciplinary Scientific Journal The Knowledge.
ISSN: 2675-9128. São Paulo-SP.

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

cost and induce both cellular and humoral responses in the host. Most of the studies on T. gondii DNA vaccines have been based on highly virulent T. gondii which possessed the dense granule proteins (GRA), surface antigens (SAG), rhoptry proteins (ROP), microneme proteins (MIC) and plant-like calcium-dependent protein kinases (CDPKs). In addition, it is worth noting that the variation in protection in mice was from increased survival and brain cyst counts.

Figure 3 - Schematization of the action of DNA vaccines in the cell.



Source: own authorship (2022).

Recombinant adenovirus vaccine

This vaccine was formed from recombinant adenoviruses that in previous studies were used to express antigens and thus used for immunization protocols. Thus, adenoviruses that could express antigens for T, gondii were studied in order to generate protection against acute and chronic toxoplasmosis in mice. Thus, we arrived at the vaccine produced with recombinant adenoviruses expressing tGMIC3, TgSAG2 or TGROP9 acts on T. gondii protein 3 (MIC3) and rhoptry 9 (ROP9) and surface antigen 2 (SAG2). These proteins and this antigen are related to the active invasion of the parasite in female BALB/c mice, and thus the recombinant adenovirus present in the vaccine should act in the production of humoral immunity, in order to produce cytokines IL-6, TNF-ÿ and IL-22, which are important for the formation of a TH17 immune response pattern that is responsible for the

RCMOS – Multidisciplinary Scientific Journal The Knowledge.
ISSN: 2675-9128. São Paulo-SP.

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

stimulation of inflammation and for the generation of humoral immunity. In addition, IF-ÿ, IL-17A and IL-10 were also used. In addition, mouse anti-ÿ-actin IgG and rabbit anti-MIC3/SAG2/ROP9-His IgG were used as primary antibodies, and goat anti-rabbit IgG as secondary antibodies. The results of the vaccine application indicated that immunization by means of this vaccine resulted in effective immune protection against T. gondii tachyzoites RH lethality by means of bivalent or trivalent recombinant adenoviruses that may have a broad stimulatory effect on cellular and humoral immune responses in BALB/c mice in addition to optimizing innate immunity. Thus, it can be seen that this vaccine, by presenting high antibody and cellular responses, is important for the study of effective vaccines against toxoplasmosis. However, the same does not show the days and if there was survival of mice, besides, it does not show if there was a reduction in parasite load, being these the negative points of this analysis. It should be noted that the route of administration of the vaccine was not described in the study (ZHANG et al., 2019).

Multi-epitope ROP8 DNA vaccine

The new DNA vaccine contains multi-epitope ROP8, which encodes the potential B- and T-cell epitopes of the ROP8 protein in order to assess the immunity and protective efficacy to combat acute T. gondii infection in BALB/c mice. In the study, this DNA vaccine was injected and the results indicated that, upon application, strong humoral and cellular responses were induced, in which a TH1 response was formed, with subsequent IFN-y production. Furthermore, the survival time of the mice was prolonged. Thus, it is suggested that the study of the selection of multiple potential epitopes may be promising and influential in the design of vaccines against acute T. gondii infection. And therefore, it becomes positive points the strong induction of immune response against toxoplasmosis. However, other data, such as the amount of days of survival that this vaccine provided to the mice, if there was a decrease in brain cysts, and if there was a reduction in parasite load, were not present in the study of this type of vaccine with ROP8, these being negative points (FOROUTAN et al., 2020).

DNA ROM4 and peptide vaccine

In this study on DNA ROM4 and peptide vaccine, a DNA peptide booster vaccination regimen was used from the identification, through a bioinformatics approach, of the polypeptide (YALLGALIPYCVEYWKSIPR), aiming to evaluate the protective efficacy of various vaccinations strategies using TgROM4. To this end, BALB/c mice were then immunized intramuscularly four times, and after immunization, enzyme-linked immunosorbent assays were used to determine IgG and cytokine productions in the mice. The results obtained showed that mice vaccinated with different immunization regimes had prolonged survival times, produced specific cellular and humoral

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

responses, thus generating high levels of IgG, IgG2a, and interferon (IFN), and, compared to negative controls, obtained a reduced number of brain cysts after T. gondii infection. With this, it becomes a positive point of the vaccine, its high rates of antibody response and cellular immune response, being a promising approach to make DNA immunization effective. However, studies on this vaccine have not presented the exact days of survival of the mice nor if there was a reduction in the parasite load, thus, these are the negative points of the use of this means of immunization (HAN et al., 2017).

pVAX-ROP54

The T. gondii parasite has an effector protein, rhoptry 54 (ROP54), which has been indicated as a virulence factor for toxoplasmosis by modulating GBP2 loading in vacuoles containing the parasite, a factor that may also be responsible for modulating aspects of the parasite. host immune response. With this information, a vaccine was created with the aim of offering protection against acute and chronic toxoplasmosis. Mice that received the vaccine had a high level of specific antibody response, lymphocyte proliferation and an increase in Th1 and Th2 cytokines, showing that pVAX-ROP54 significantly induces cellular-like responses mediated by T lymphocytes. cytotoxic and humoral effects, mediated by B lymphocytes, in addition to extending the survival time and reducing the burden level of brain cysts by 35.9%. Therefore, immunizing with the recombinant ROP54 plasmid may provide partial protection against acute and chronic toxoplasmosis. The vaccine was administered intramuscularly. YANG *et* al., 2017.

pcROM4 + pcGRA14 Vaccine

This vaccine was composed of a DNA peptide booster in order to evaluate the protective efficacy of prophylaxis with TgROM4 in the composition. Thus, it was noticed that the rhomboid 4 (ROM4) and DNA cocktail (ROM4 + GRA14) vaccines of the RH strain of the T. gondii parasite coated with calcium phosphate nanoparticles (CaPNs) considerably increased cellular immune responses, which depend on of cytotoxic T lymphocytes and humoral immune responses, characterized by the participation of specific antibodies found in the blood plasma of mice. These responses, then, have an action on the parasite, when compared with pcROM4 and the DNA cocktail vaccine without CaPNs, lead to an increase in the survival time of mice, in addition to increasing immune responses and protection against acute toxoplasmosis. HAN *et* al., 2017.

pcGRA14 + rGRA14 + CaPNs Vaccine

The potential vaccine is produced from the dense granule antigen 14 (GRA14), together with

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

calcium phosphate booster (CaPNs) or aluminum hydroxide (Alum) nano-adjuvants and was designed with the aim of partial or total protection for infection by T.gondii. Prime-boost immunization using recombinant protein and plasmid DNA of the dense granule antigen 14, rGRA14 and pcGRA14, respectively, with nano-adjuvants obtained significant levels of specific IgG and cytokines. Mice from the rGRA14-CaPNs and pcGRA14 + rGRA14-CaPNs immunization groups showed high levels of total IgG, IgG2a and IFN-ÿ, confirming a Th1-type response. On the other hand, mice in the rGRA14-Alum and pcGRA14 + rGRA14 immunization groups culminated in specific levels of IgG1 and IL-4, confirming a Th2-type response. Mice that were immunized with this DNA primary protein booster vaccine with nano-adjuvants showed stronger responses than those immunized with other combinations of antigens. Furthermore, mice from the CaPNs group showed longer survival time and lower burden of brain cysts than the other group, in addition to dramatically increasing humoral immune responses, characterized by the participation of specific antibodies found in blood plasma and cellular, mediated immune responses. by cytotoxic T lymphocytes. Therefore, the results demonstrate that this vaccine may be promising against T.gondii infection. PAGHEH *et* al., 2019.

pVAX-ROP35

The TgROP35 DNA composition was used to evaluate the protective effect of this vaccine in experimental mice challenged with T. gondii. Thus, after vaccination of the mice with pVAX-ROP35, a significant increase in the levels of IgG, IgG1, IgG2, IFN-ÿ, IL-2 and IL-10 was noticed, but the expression of IL-4 did not show any difference between the vaccinated and control (unvaccinated) groups. In addition, there was a prolongation in the survival of mice after infection with the virulent strain of T. gondii RH compared to unvaccinated control animals, and also a decrease in the number and size of brain cysts. Thus, the vaccine generated an increase in cellular immunity responses, mediated by cytotoxic T lymphocytes and also in humoral responses, induced by specific antibodies formed by B lymphocytes of the immune system. Furthermore, it was noted that the defense mechanisms against T. gondii were partially induced after vaccination. ZHANG *et* al., 2018.

Multi-antigen DNA vaccine (Psag5b/SAG5C)

Surface antigen proteins SAG5B and SAG5C are potential stimulators of cellular and humoral immune responses against T. gondii infections, which occur worldwide and can lead to congenital infections in humans.

The multi-antigenic DNA vaccine was designed to express the T. gondii surface antigen proteins 5B and 5C (SAG5B and SAG5C) simultaneously, which are potential stimulators of cellular

RCMOS – Multidisciplinary Scientific Journal The Knowledge.
ISSN: 2675-9128. São Paulo-SP.

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

and humoral immune responses. The group of mice that received the multi-antigen DNA vaccine (pSAG5B/SAG5C) obtained a higher production of IFN-ÿ and IgG antibodies, especially IgG2a, than the group of mice that received the single DNA vaccine (pSAG5B or pSAG5C) or the control group (do not vaccinate). In addition, the mice vaccinated with pSAG5B +SAG5C obtained a longer survival time and a lower number of brain cysts when compared to the other groups. Therefore, the multi-antigen DNA vaccine induces significant immune responses and improves protection against T. gondii infection. The site of vaccine administration was not indicated (LU et al., 2017).

PIRESneo/MIC6/TgPLP1

The potential vaccine is a DNA vaccine using a eukaryotic plasmid (pIRESneo) expressing micronemal protein 6 (MIC6) and perforin-like protein 1 (TgPLP1), which are closely linked to T. gondii host cell invasion. Parasites deficient in TgPLP1 of T. gondii were trapped inside the host cells, as they were unable to exit the cells normally.

After the vaccine was injected intramuscularly into the mice, it was realized that the fusion of TgPLP1 with MIC6 generated a strong Th1 cellular and humoral response, with significant levels of IgG against T. gondii, in addition to lymphoproliferation. Therefore, a decrease in mortality and an increase in survival was also observed in these mice.

On the other hand, a DNA vaccine expressing only one gene is not able to induce a total response, only partial, making it necessary to create a vaccine expressing more genes and thus making the process of its creation more difficult (YAN et al., 2012).

SAG5A Vaccine

The antigenic determinants that could be related to T and B cells were identified, which allowed the construction of the SAG5A vaccine, which is constituted of DNA (LU et al., 2015). The administration of the immunizer occurred in mice, together with peptide, and significant cellular and humoral immune responses were elicited (LU et al., 2015). In addition, with the application of the immunizer there was a 35% reduction in brain cysts arising from toxoplasmosis in these mice (LU et al., 2015).

pVAX-ROP5 and pVAX-GRA15 vaccine

The use of the Toxoplasma gondii ROP5 protein 5 as well as the GRA15 antigen for the construction of eukaryotic plasmids (pVAX-ROP5 and pVAX-GRA15 respectively) in the immunization of Kunmig mice has been shown to be effective both when used separately and when

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

used together. When administered one or the other, the mice showed an increase in survival time of about 19.4 ± 4.9 days for ROP5 and 17.8 ± 3.8 days for GRAP15; there was a significant increase in serum IgG2a titer in addition to the combination of Th1 responses that enabled increased levels of IFN-ÿ, IL-2, IL-12 p70 and IL-12 p40. In addition, an increase in TCD8+ cells acting with cytotoxicity and a reduction in brain cysts of 57.4% for ROP5 and 65.9% for GRAP15. In relation to the joint administration of both immunizers there is a more significant immune response, both cellular and humoral, highlighting the increase in survival time to 22.7 ± 7.2 days and reduction of brain cysts to 79%. (CHEN et al., 2015).

pVAX-ROP38 Vaccine

The efficacy of the pVAX-ROP38 vaccine in female Kunmig mice, which acts to encode Toxoplasma gondii protein 38, was shown to be extremely effective in cases of chronic infections. The administration of three intramuscular injections of the immunizer, two weeks apart, triggered a significant cellular and humoral response. There was a considerable increase in T lymphoproliferative response and IgG antibodies as well as increased IgG2a/IgG1 activation of Th1 response with increased IFN-ÿ and IL-2. CD4+ and CD8+ cell levels were also elevated in addition to a 76.6% reduction in brain cysts in models of chronic infection when compared to the control group using PBS or pVAX I. It is noteworthy that in acute infection models there was no significant difference in the survival time of the control group compared to mice protected with pVAX-ROP38. (XU et al.,2014).

Prime-boost vaccine with TgPI-1

The use of serine protease-1 inhibitor immunizer (TgPI-1) in C57BL/6 mice (highly susceptible) by intradermal and intranasal coadministration showed promising results. The applicability of alum-associated rTgPI-1 (intradermally) added to the action of CpG-ODN-associated rTgPI-1 (intranasally), which acted as a booster, triggered specific humoral Th1/Th2 response in the ileum as well as increased mucosal immune response through specific IgA and positive cellular response in mesenteric lymph node cells. This prime-boost vaccination technique showed a greater than 60% reduction in brain parasite load in mice tested with cystogenic Me49 T. gondii strain; and demonstrated that there is a possibility of protection to other unimmunized mice by adoptive transfer of mesenteric lymph nodes from those vaccinated. (SANCHEZ et al., 2015).

pVAX-RON5p

The immunizer was based on a recombinant plasmid pVAX-RON5p, which is associated with

RCMOS – Multidisciplinary Scientific Journal The Knowledge.
ISSN: 2675-9128. São Paulo-SP.

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

the possibility of expression of Rhoptry neck protein 5 (RON5) in eukaryotes, this protein being associated with T. gondii invasion (ZHAO; LI; CHEN; SUN; LIU; ZHU; ZHOU, 2016). The immunization in question was a trigger of humoral and cellular immune responses in BALB mice facing acute infections, in which an increase in survival time after infection was noted, and chronic infections by T. gondii, in which a reduction in the number of cysts in immunized BALB were identified (ZHAO; LI; CHEN; SUN; LIU; ZHU; ZHOU, 2016). Thus, it is concluded that the RON5p DNA vaccine is able to trigger partial protective immunity in the face of T. gondii infection (ZHAO; LI; CHEN; SUN; LIU; ZHU; ZHOU, 2016).

Multi-antigenic DNA vaccine expressing pROP5/ROP7

Knowing that the ROP5 and ROP7 proteins are potential stimulators of cellular and humoral immunity, a multi-antigenic immunizer was produced with the two proteins (WANG; LU; ZHOU; HAN; GUO; ZHOU; CONG; HE, 2016). Immunization was performed three times intramuscularly in mice (WANG; LU; ZHOU; HAN; GUO; ZHOU; CONG; HE, 2016). Compared to mice immunized with a gene alone, there was a 4-5 day increase in survival (WANG; LU; ZHOU; HAN; GUO; ZHOU; CONG; HE, 2016). In addition, a reduction in the number of brain cysts was noted with this immunizer, and it was associated with an important increase in protection against T. gondii (WANG; LU; ZHOU; HAN; GUO; ZHOU; CONG; HE, 2016).

DNA vaccine inducing TgROP17

The developed immunizer encodes the TgROP17 protein and was injected into BALB mice intramuscularly (WANG; WANG; PEI; BAI; YIN; GUO; YIN, 2016). Thereafter, induction of humoral immunity was noted, with high rates of anti-T. gondii antibodies, with the presence of IG1 and, predominantly, IG2a, in addition to induction of cellular immunity, with high lymphocytes, being associated with Th1-type response (WANG; WANG; PEI; BAI; YIN; GUO; YIN, 2016). There was increased survival time, being considered the propitious immunizer in fighting acute toxoplasmosis (WANG; WANG; PEI; BAI; YIN; GUO; YIN, 2016).

Recombinant protein and subunit vaccine

Recombinant protein and subunit vaccines are extremely safe and have a low chance of harm effects due to the incorporation of a highly purified antigen as the main component of the vaccine. On the other hand, like DNA vaccines, a specific antigen involved in T. gondii disease must be identified in order to be used.

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

Irradiated extracts of T. gondii tachyzoite antigens have been found to have the ability to prime the immune system with a potent immunity, both humoral and cellular, capable of producing protection against the RH and ME49 strains in mice. Irradiated soluble T. gondii antigens conferred, with high dose radiation, a similar level of protection to irradiated RH tachyzoites. Both had identical levels of peripheral B-lymphocyte induction in blood and survival, but on the other hand, the irradiated soluble antigen induced a higher percentage of TCD4+ lymphocytes, while the irradiated HR tachyzoites induced a higher percentage of TCD8+ lymphocytes. Vaccines designed using the lysed T. gondii antigens adjuvanted with QuilA have had their efficacy proven in pigs, consistent with the findings in mice, in which they reduced the parasite load in muscle tissues.

Interesting results have also been reported for subunit vaccines that express proteins associated with the stress response. The peroxiredoxin 1-based vaccine produced cytokines IL-12 and IL-6, promoting survival of mice against the PLK strain (Ki-Back Chu and Fu-Shi Quan, 2021).

Nanoparticle vaccine

Nanoparticle vaccines have recently emerged, not only to combat T. gondii infection, but also to be a carrier for several other vaccines. These vaccines protect antigens that normally undergo proteolytic degradation and thus facilitate their uptake by antigen-presenting cells (APCs) for the induction of the immune response. Although recent, studies indicate that these vaccines are highly effective.

The adjuvanted multiepitope nanoparticle vaccine with GLA-SE-stimulated IFN-gamma secretion conferred protection against T. gondii. Uniformly, the chimeric polypeptide vaccine in which expresses TCD4 lymphocytes, TCD8 lymphocytes and B lymphocyte epitopes emulsified in GLA-SE conferred protection to mice.

Maltodextrin-based nanoparticle vaccines formulated with T.gondii antigen extracts were administered intranasally and conferred high protection against chronic and congenital toxoplasmosis (Ki-Back Chu and Fu-Shi Quan, 2021).

Vaccines from virus-like particles (VLPs)

Vaccines from virus-like particles (VLPs) are a new method of vaccines. They are devoted to the genetic material necessary for replication, making them completely safe, can migrate quickly to lymph nodes due to their size, and repetitively present the particle's surface antigens, promoting a rapid and potent induction of the immune response. But despite this, their study is limited.

Several studies have been conducted investigating the efficacy of VLPs vaccines using well-characterized T. gondii antigens such as ROP4 and ROP13, in which they completely protected mice

RCMOS - Multidisciplinary Scientific Journal The Knowledge.
ISSN: 2675-9128. São Paulo-SP.

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

from a lethal dose of cysts and obtained a relief of inflammatory responses in the mice brains. Their efficacy was increased when these two antigens were expressed in a single VLP, as it led to a 100% survival rate among the mice, and also dramatically reduced the amount of the cysts. Multigenic VLPs expressing BMI, ROP18 and MIC8 had a similar finding. On the other hand, VLPs expressing the AMA1 proteins were not as effective.(Ki-Back Chu and Fu-Shi Quan, 2021)

Attenuated T. gondii vaccine

Attenuated T. gondii vaccines, although safety concerns remain, are highly effective and confers nearly complete protection against many strains of T. gondii compared with other forms of vaccine. Furthermore, most attenuated T. gondii vaccines have been made targeting the parasite's biosynthetic pathways and have shown high protection against acute, chronic and congenital toxoplasmosis when tested in mice and other animals, such as felines. Therefore, the vaccine may be promising in preventing all stages of T. gondii infection. (Ki-Back Chu and Fu-Shi Quan, 2021)

The potential vaccine is produced from ultraviolet attenuated T. gondii together with pidotimod, in which is a synthetic substance capable of stimulating human dendritic cells to release large amounts of pro-inflammatory molecules and furthermore induce T-cell proliferation and differentiation towards a Th1 phenotype, ie it is able to induce cellular and humoral immunity. After infection of the test group mice with 100 tachyzoites, they were immunized intraperitoneally and showed higher survival rate and time (9 to more than 30 days), lower parasite load and less damage liver histopathology, besides producing high levels of Th1 type cytokine (IL-2, IFN-gamma and TNF-alpha) and anti-toxoplasma IgG compared to the other group.

On the other hand, this vaccine is not yet suitable for human use because there is a high chance of reactivation of the parasite to the pathogenic form.

Therefore, besides pidotimod being safe and easy to use for humans, it can still induce the Th1 response and may provide an additional benefit in a future vaccine against toxoplasmosis (ZHAO et al., 2013).

Carbohydrate Vaccine

Carbohydrate vaccines containing glycosylphosphatidylinositol (GPI) glycoconjugates have shown some advantages over attenuated or inactivated vaccines, as they are safer and are considered potential vaccines. Furthermore, mutations in key biosynthetic genes can cause devastating consequences for the parasite, and resistance against the carbohydrate antigens is not likely. Although such vaccines are extremely rare, some studies have shown that the vaccine did not confer adequate

RCMOS - Multidisciplinary Scientific Journal The Knowledge.
ISSN: 2675-9128. São Paulo-SP.

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

protection against T. gondii. However, further studies are being done looking at methods by which to increase the efficacy and immunogenicity of the vaccines so that, in the future, they may be an alternative vaccine against T. gondii infection (Ki-Back Chu and Fu-Shi Quan, 2021).

Table 1 - Overview of the action of DNA vaccines on T. gondii. All studies were evaluated, but some did not describe this information. +, weak; ++, intermediate; +++, strong; -, undetermined. Survival reports to survival after immunization.

Vaccine platform **(DNA)**	*Animal model*	*Antibody response*	*Cellular immune response*	*Parasite load reduction*	*Survival*	*Reference*
Recombinant Adenovirus	***Mouse***	**++**	**++**	**-**	**-**	***24***
Recombinant CDPK3	***Mouse***	**+++**	**++**	**++**	**-**	***19***
ROP8	***Mouse***	**-**	**+++**	**-**	**-**	***5***
ROM4 and Peptide	***Mouse***	**+++**	**+++**	**-**	**-**	***6***
ROP54	***Mouse***	**+**	**++**	**+**	***HR: 15 days***	***23***
ROM4+ GRA14 + CaPNs	***Mouse***	**++**	**++**	**++**	***RH: >8 days***	***6***
peGRA14+ rGRA14 + CaPNs	***Mouse***	**++**	**+++**	**++**	***HR: >8 days***	***12***

RCMOS – Multidisciplinary Scientific Journal The Knowledge.
ISSN: 2675-9128. São Paulo-SP.

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

ROP35	***Mouse***	**++**	**+++**	**+**	***HR: 14 days***	***25***
Psag5b/SAG5W	***Mouse***	**++**	**++**	**+++**	***HR: 17 days***	***10***
pIRESneo/MIC6/TgPLPI	***Mouse***	**+++**	**+++**	**++**	**-**	***22***
SAG5A	***Mouse***	**++**	**++**	**+**	**-**	***9***
ROP5+ GRA15	***Mouse***	**+++**	**+++**	**+++**	***HR: 23 days***	***2***
ROP38	***Mouse***	**++**	**++**	**+++**	**-**	***21***
TgPI-1+ Prime Boost	***Mouse***	**++**	**++**	**++**	**-**	***15***
RON5p	***Mouse***	**++**	**+**	**+**	**-**	***28***
ROP5/ROP7	***Mouse***	**+**	**+**	**+**	***RH: 4-5 days***	***17***
TgROP17	***Mouse***	**++**	**++**	**++**	**-**	***18***

Source: own authorship (2022).

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

CONCLUSION

The superiority of DNA vaccines, against toxoplasmosis, over the others until now, is due to their efficiency, ease of production, low cost and also induction of immune response. With this, and the need for comparison between these vaccines, a method was used to present their differences, benefits and disadvantages. For this, we compared the antibody response, immune response, parasite load reduction and survival of the mice used for these tests. The vaccines with calcium-dependent protein kinases (CDPK3), the vaccine composed of the junction of MIC3/ROP9/SAG2 and the mitochondrial rhomboid proteases (ROM4) added to a peptide were found to have the highest immune response (both humoral - IgG and IgM, and cellular). Furthermore, most of the studied vaccines showed medium or high humoral and cellular immune response, proving the effectiveness for immunity formation. Furthermore, the only vaccine showed that high parasite load reduction was the one composed of surface antigens (SAG5B/SAG5C), and this also showed, consequently, higher survival of mice compared to control mice (RH: 17 days). In addition, there were vaccines that did not verify in their studies the amount of parasite load reduced nor the exact days of survival of the mice, thus showing the need for further studies. Finally, according to the systematic review presented, the DNA vaccine that showed greater effectiveness in protecting against toxoplasmosis was the one instigated in mice by the mixture of surface antigens SAG5B and SAG5C.

REFERENCES

1. BARBOSA, Helene Santos; MUNO, Renata Morley de; MOURA, Marcos de Assis. **The Evolutionary Cycle. In: TOXOPLASMOSIS and Toxoplasma gondii.** Rio de Janeiro: Fiocruz, 2014. p. 33-45. Available at: https://books.scielo.org/id/p2r7v/pdf/souza-9788575415719-04.pdf . Accessed on: February 20, 2022.

2. Chen J, Li ZY, Petersen E, Huang SY, Zhou DH, Zhu XQ. **DNA vaccination with genes encoding Toxoplasma gondii ROP5 and GRA15 antigens induces protective immunity against toxoplasmosis in Kunming mice.** Vaccines Rev Expert. 2015 Apr;14(4):617-24. doi: 10.1586/14760584.2015.1011133. PMID: 25749394.

3. Chu, Ki-Back, and Fu-Shi Quan. 2021. **"Advances in Toxoplasma gondii Vaccines": Current Strategies and Challenges for Vaccine Development"** Vaccines 9, no. 5: 413. https://doi.org/10.3390/vaccines9050413

4. DODANGEH, Samira; DARYANI, Ahmad; SHARIF, Mehdi; AGHAYAN, Sargis A.; PAGHEH, Abdol Satar; SARVI, Shahabeddin; PRAY, Fatemeh. **A systematic review on efficiency of microneme proteins to induce protective immunity against Toxoplasma gondii.** European Journal Of Clinical Microbiology & Infectious Diseases, [SL], v. 38, no. 4, p. 617-629,

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

24 Jan. 2019. Springer Science and Business Media LLC. http://dx.doi.org/10.1007/s10096-018-03442-6.

5. Foroutan M, Ghaffarifar F, Sharifi Z, Dalimi A. **Vaccination with a novel multiepitope ROP8 vaccine against acute Toxoplasma gondii infection induces strong B and T cell responses in mice.** Dis. Immunolem Microbiol Comp. 2020 Apr;69:101413. doi: 10.1016/j.cimid.2020.101413. Epub 2020 Jan 8. PMID: 31954995.

6. Han Y, Zhou A, Lu G, Zhao G, Wang L, Guo J, Song P, Zhou J, Zhou H, Cong H, He S. **Protection through a DNA and peptide ROM4 vaccine against Toxoplasma gondii in BALB/c mice.** BMC Infect Dis. 2017 Jan 11;17(1):59. doi: 10.1186/s12879-016-2104-z. PMID: 28077075; PMCID: PMC5225637.

7. LANNES-VIEIRA, Joseli. **Immune Response in Toxoplasma gondii Infection: challenges and opportunities.** In: TOXOPLASMOSIS and Toxoplasma gondii. Rio de Janeiro: Fiocruz, 2014. p. 83-98. Available at: https://books.scielo.org/id/p2r7v/pdf/souza-9788575415719-08.pdf. Accessed on: February 20, 2022.

8. LOVISON, Robson; RODRIGUES, Renata Mendonça. **INCIDENCE AND PREVALENCE OF TOXOPLASMOSIS IN THE SOUTHERN REGION OF BRAZIL: A LITERATURE REVIEW.** Rev. Saúde Públ. Santa Cat, Florianópolis, v. 10, n. 3, p. 61-75, Sept. 2017.

9. LU, Gang; WANG, Lin; ZHOU, Aihua; HAN, Yali; GUO, Jingjing; SONG, Pengxia; ZHOU, Huaiyu; CONG, Hua; ZHAO, Qunli; HE, Shenyi. **Epitope analysis, expression and protection of SAG5A vaccine against Toxoplasma gondii.** Acta Tropica, [SL], v. 146, p. 66-72, jun. 2015. Elsevier BV. http://dx.doi.org/10.1016/j.actatropica.2015.03.013.

10. Lu G, Zhou J, Zhou A, Han Y, Guo J, Song P, Zhou H, Cong H, Hou M, Wang L, He S. **The SAG5B and SAG5C combined vaccine protects mice against Toxoplasma gondii infection.** Parasitol Int. 2017 Oct;66(5):596- 602. doi: 10.1016/j.parint.2017.06.002. Epub 2017 Jun 8. PMID: 28602862.

11. NEVES, DP et al. **Human Parasitology.** 12th ed. São Paulo: Atheneu, 2011.

12. Pagheh AS, Sarvi S, Gholami S, Asgarian-Omran H, Valadan R, Hassannia H, Ahmadpour E, Fasihi-Ramandie M, Dodangeh S, Hosseni-Khah Z, Daryani A. **Protective efficacy induced by vaccination with Toxoplasma gondii GRA14 in mice with DNA prime and recombinant protein boost.** Microb Pathog. doi: 10.1016/j.micpath.2019.103601. Epub 2019 Jun 15. PMID: 31212035. 2019 Sep;134:103601.

13. PINZAN, Camila Figueiredo *et al.* **Vaccination with Recombinant Microneme Proteins Confers Protection against Experimental Toxoplasmosis in Mice. Plos One,** [SL], v. 10, no. 11, p. 0143087, 17 Nov. 2015. Public Library of Science (PLoS). http://dx.doi.org/10.1371/journal.pone.0143087.

14. Rahimi MT, Sarvi S, Sharif M, Abediankenari S, Ahmadpour E, Valadan R, Ramandie MF,

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

Hosseini SA, Daryani A. **Immunological evaluation of a DNA cocktail vaccine with co-delivery of calcium phosphate nanoparticles (CaPNs) against the** Toxoplasma gondii RH strain in BALB/c mice. Parasitol Res. 2017 Feb;116(2):609-616. doi: 10.1007/s00436-016-5325-6. Epub 2016 Dec 2. PMID: 27909791.

15. Sánchez VR, Fenoy IM, Picchio MS, Soto AS, Arcon N, Goldman A, Martin V. **Homologous prime-boost strategy with TgPI-1 enhances immune response and protects highly susceptible mice against chronic Toxoplasma gondii infection.** Acta Trop. 2015 Oct;150:159-65. doi: 10.1016/j.actatropica.2015.07.013. Epub 2015 Jul 19. PMID: 26200784.

16. VISSER, Silvia; GIATTI, Leandro Luiz; CARVALHO, Ricardo Augusto Chaves de; GUERREIRO, Jose Camilo Hurtado. **Study of the association between socioenvironmental factors and the prevalence of intestinal parasitosis in a peripheral area of the city of Manaus (AM, Brazil).** Ciência & Saúde Coletiva, [SL], v. 16, n. 8, p. 3481-3492, Aug. 2011. FapUNIFESP (SciELO). http://dx.doi.org/10.1590/s1413-81232011000900016.

17. WANG, L.; LU, G.; ZHOU, A.; HAN, Y.; GUO, J.; ZHOU, H.; CONG, H.; HE, S.. **Evaluation of immune responses induced by rhoptry protein 5 and rhoptry protein 7 DNA vaccines against Toxoplasma gondii.** Parasite Immunology, [SL], v. 38, no. 4, p. 209-217, 30 March. 2016. Wiley. http://dx.doi.org/10.1111/pim.12306.

18. WANG, Hai-Long; WANG, Yu-Jing; PEI, Yan-Jiang; BAI, Ji-Zhong; YIN, Li-Tian; GUO, Rui; YIN, Guo-Rong. **DNA vaccination with a gene encodingToxoplasma gondiiRhoptry Protein 17 induces partial protective immunity against lethal challenge in mice.** Parasite, [SL], v. 23, no. 4, p. 1-9, Feb. 2016. EDP Sciences. http://dx.doi.org/10.1051/parasite/2016004.

19. Wu M, An R, Chen Y, Chen T, Wen H, Yan Q, Shen J, Chen L, Du J. **Vaccination with recombinant Toxoplasma gondii CDPK3 induces protective immunity against experimental toxoplasmosis.** Acta Trop. 2019 Nov;199:105148. doi: 10.1016/j.actatropica.2019.105148. Epub 2019 Aug 16. PMID: 31425673.

20. XIA, Ningbo; ZHOU, Taifang; LIANG, Xiaohan; Ye, Shu; ZHAO, Pengfei; YANG, Jichao; ZHOU, Yanqin; ZHAO, Junlong; SHEN, Bang. **A Lactate Fermentation Mutant of Toxoplasma Stimulates Protective Immunity Against Acute and Chronic Toxoplasmosis.** Frontiers In Immunology, [SL], v. 9, p. 1-13, 10 Aug. 2018. Frontiers Media SA. http://dx.doi.org/10.3389/fimmu.2018.01814.

21. Xu Y, Zhang NZ, Tan QD, Chen J, Lu J, Xu QM, Zhu XQ. **Evaluation of the immuno-efficacy of a novel DNA vaccine encoding Toxoplasma gondii rhoptry protein 38 (TgROP38) against chronic toxoplasmosis in a murine model.** BMC Infect Dis. 2014 Sep 30;14:525. doi: 10.1186/1471-2334-14-525. PMID: 25267356; PMCID: PMC4261603.

22. YAN, Hai-Kuo et al. **Vaccination with a DNA Vaccine Coding for Perforin-Like Protein 1 and MIC6 Induces Significant Protective Immunity against Toxoplasma gondii.** Clinical And Vaccine Immunology, [SL], v. 19, no. 5, p. 684-689, 29 Feb. 2012. American Society for Microbiology. http://dx.doi.org/10.1128/cvi.05578-11.

Year V, v.2 2025 | Submission: 08/12/2025 | Accepted: 10/12/2025 | Publication: 12/12/2025

23. Yang WB, Zhou DH, Zou Y, Chen K, Liu Q, Wang JL, Zhu XQ, Zhao GH. **Vaccination with a DNA vaccine encoding Toxoplasma gondii ROP54 induces protective immunity against toxoplasmosis in mice.** Acta Trop. 2017 Dec;176:427-432. doi: 10.1016/j.actatropica.2017.09.007. Epub 2017 Sep 18. PMID: 28935555.

24. Zhang D, Jiang N, Chen **Q. Vaccination with recombinant adenoviruses expressing Toxoplasma gondii MIC3, ROP9, and SAG2 provides protective immunity against acute toxoplasmosis in mice.** Vaccine. 2019 Feb 14;37(8):1118-1125. doi: 10.1016/j.vaccine.2018.12.044. Epub 2019 Jan 19. PMID: 30670302.

25. Zhang Z, Li Y, Liang Y, Wang S, Xie Q, Nan X, Li P, Hong G, Liu Q, Li X. **Molecular characterization and protective immunity of Toxoplasma gondii protein 35 (ROP35) as a vaccine against DNA.** Parasitol Vet. 2018 Aug 30;260:12-21. doi: 10.1016/j.vetpar.2018.06.016. Epub 2018 Jun 24. PMID: 30197008.

26. ZHANG, Nian-Zhang; Xu, Ying; WANG, Meng; CHEN, Jia; HUANG, Si-Yang; GAO, Qi; ZHU, Xing-Quan. Vaccination with Toxoplasma gondii calcium-dependent protein kinase 6 and rhoptry protein 18 encapsulated in poly(lactide-co-glycolide) microspheres induces long-term protective immunity in mice. **Bmc Infectious Diseases,** [SL], v. 16, no. 1, p. 1-11, 18 Apr. 2016. Springer Science and Business Media LLC. http://dx.doi.org/10.1186/s12879-016-1496-0.

27. Zhao, Y., Huang, B., Huang, S. et al. **Evaluation of the adjuvant effect of pidotimod on immune protection induced by UV-attenuated Toxoplasma gondii in mouse models.** Parasitol Res 112, 3151-3160 (2013). https://doi.org/10.1007/s00436-013-3491-3

28. ZHAO, Yu; LI, Zhong-Yuan; CHEN, Jia; SUN, Xiao-Lin; LIU, Shan-Shan; ZHU, Xing-Quan; ZHOU, Dong-Hui. Protective efficacy of pVAX-RON5p against acute and chronic infections of Toxoplasma gondii in BALB/c mice. **Experimental Parasitology,** [SL], v. 163, no. 1, p. 24-30, apr. 2016. Elsevier BV. http://dx.doi.org/10.1016/j.exppara.2016.01.011.