# Vaccines against Ovine Toxoplasmosis: History, Advances and Current status

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## Abstract

**Toxoplasmosis is a common and wide spread global disease, caused by a protozoan parasite, *Toxoplasma gondii*. The condition afflicts both animal and human, causing abortion and congenital defects. Hence, it is of a huge veterinary and medical concern. With on-going researches, the mechanism of the disease is better appreciated nowadays. Developing a vaccine for veterinary use, more precisely for sheep will not only gain economic advantages, but it will also bring insight into discovering one for human use as well. Until now, the only available commercial vaccine in sheep is based on the attenuated *T.gondii* strain S48, known as Toxovac®, but the vaccine is only capable to provide a short immunity. Additionally, it is expensive and inappropriate to be applied in human. In recent years, different experimental studies have demonstrated the probability of developing a new vaccine. This paper tries to highlight the history of discovering the first commercial vaccine against toxoplasmosis in sheep, along with recent advances in the field and shining a light to the current status of vaccine trials.**

**Keywords:** Toxoplasmosis, Immunity against *T.gondi*, Vaccines in sheep, DNA vaccines.

## Introduction

Toxoplasmosis is a parasitic disease caused by an intracellular parasite *Toxoplasma gondii*. The disease affects both animal and human [1,2]. Common problems associated with toxoplasmosis are infection of pregnant animals that causes abortion, early neonatal death and deformities in sheep, which results in huge economic losses [3]. In addition, the parasite can reside or localize in the muscle tissues of sheep in cyst form and then transfer to human through their consumption of under-cooked or raw meat, which is of high risk to the foetus as well as to the immunocompromised patients [1,4].

The final host of the parasite is feline (cat family), the pathogen is also able to shed in a form of unsporulatedoocysts, which then pass via faeces and become sporulated in the environment and are then able to infect it's intermediate hosts such as animal and human [5]. Moreover, the parasite is capable of transmitting among its intermediate hosts and can be found in two stages, which could be either tachyzoites (stage of multiplication) or bradyzoites (tissue cyst) [6]. The tachyzoites are sporozoites, released by ingested or localized sporulatedoocysts in the small intestine (Mesenteric lymph node), which eventually causes parasitemia.

In blood circulation, the parasite travels to many tissues including the brain and muscle tissue in bradyzoites form which are enclosed in a cyst [4]. In pregnant ewes, after establishing parasitemia, the parasite invades placenta and cause abortion. Thus, a strict and regular prevention strategy is essential to control the spread and development of the disease [1,7]. Developing a successful vaccine in sheep is not only important for preventing abortion in pregnant ewes, but also it can pave away for finding a good vaccination approach in human as well.

## I. Immunity against *T.gondii*

It is important to understand the mechanism by which hosts respond to the pathogen during infection in order to address the best possible approach for vaccination.

Shortly, after ingestion of the pathogen and establishment of parasitaemia, the tachyzoites of *T.gondii* are recognized by phagocytic cells through interaction between actin-binding protein named profilin, which is a ligand for Toll-like receptors, in particular TLR2, TLR4 [8] and (TLR11) [9]. On dendritic cells, TLR11 recognizes perforin-like molecule that present on the surface of the parasite, which then signals via MyD88 pathway, eliciting the synthesis of various cytokines mainly IL-12, IFN-γandTNF-α. Additionally, the *T.gondii*cyclophilin, which is a protein binding molecule, provokes synthesis of IL-12 via Cystine Chemokine Receptor 5 (CCR5) on dendritic cells [2,10]. Both IFN-γandIL-12 stimulate Th1 response, resulting in production of antibodies, which aid complement system to eliminate tachyzoites circulating in the blood stream [11].

As the parasite can enter and survive inside the cells, soon after establishing of infection, IL-12 triggering Th1 response aids in the destruction of intracellular tachyzoites [2,12]. The mechanism is found to be through releasing of IFN-γ, the latter acts synergistically with TNF- α to mediate killing process by macrophages through formation of phagolysosome, and by the action of nitrous oxide and reactive oxygen intermediates [12].

In most circumstances, the engulfed tachyzoites release different proteins such as microneme and dense granule antigens. The microneme contains a proteinaceous structure termed perforin-like molecule which disrupts phagosome formation [13]. As the parasite starts to replicate inside the phagocytic cells, calcium ion concentration begins to increase [14]. This elevation together with action of perforin like molecules allow T. gondii to egress and infect other cells using an adhesive based motility named gliding. On this occasion, the infected phagocytic cells are recognized by T cells through antigen presentation via MHC I and MHC II [15]. Ultimately, it results in the production of cytokines (TNF- α and IFN-γ) by CD8+ and CD4+ T cells [16]. Also both TNF- α and IFN-γ aids the conversion of the parasite from tachyzoites to bradyzoites [17].

## II. Vaccine Candidates

### *A. Vaccines based on the T.gondii strains*

The sentinel activity resulted from a natural infection proposed that the development of an effective vaccine is highly possible. In spite of many attempts and crucial progress toward the understanding of the immune responses occurring following infection by *T.gondii*, there are still no convincing immune prevention methods against toxoplasmosis.

#### 1. Commercially available vaccine Strain S48, Toxovax®)

The first commercial vaccine in sheep was developed and marketed in New Zealand in 1988 [18]. Following this, it was further

assessed and launched in the UK in 1992 [19]. The vaccine comprises of attenuated tachyzoites of T. gondii Strain48 (Toxovax®) concentrated in aqueous suspension [18]. It was first isolated from dead or aborted lambs in New Zealand. At that time, researchers demonstrated that over time, the parasite lost its ability to develop to bradyzoites after passing more than 3000 times in mice before applying it in sheep. Dilution of the vaccine is needed prior to use and the preferred site of injection is subcutaneous region of the neck [18].

The S48 can establish a short infection prior to elimination by the immune system. After injection, the S48 (Toxovax®) begins to proliferate in the draining lymph nodes resulting in a moderate degree of fever between day 5 and 10.

Throughout this time, it is possible to detect tachyzoites in the blood stream [19,20]. Then after 10 days, CD4+ T cells predominate. In addition to production of antibody which reaches a peak titre in 6 weeks [13,19,21]. Following this period, the cell mediated immune response shifts to CD8+ T cells, with production of high amounts of IFN-γ. After that, the antibody titre starts to decline gradually until the week 20. Therefore, the parasite cannot produce a long term infection [21].

The S48 strain vaccine has several advantages. Frist of all, when a vaccinated sheep expose to the *T.gondii*, the parasite is restricted to the mesenteric lymph node and it loses the ability to penetrate and infect the placenta (Gravid uterus). Hence, it cannot induce abortion. A further advantage is involved in the prevention of tissue cyst formation through avoiding development of bradyzoite in muscle tissues; this point is of great importance for preventing zoonotic risks. Moreover, one shot of the vaccine is sufficient to induce immune response [19, 21,22].

On the other hand, the S48 has a number of drawbacks. Above all, it has a short shelf life 7-10 days, and it can cause infection in humans through handlers during vaccine administration. What is more; the vaccine is expensive in terms of price and beside this the sentinel effect of the vaccine lasts for 18 months, after that period, again bradyzoites in muscle tissues may turn into a cyst form [19].

## 2. ME49 Strain

A number of experiments have been conducted to evaluate the possibility of using low virulence ME49 strain as a vaccine which is originally isolated from muscle of sheep [23]. This strain exhibited to be protective in mice, the mechanism was found to be through producing high levels of IL-12, IL-6, TNF-α and IFN-γ which are crucial for elimination of the pathogen within the host cells [24]. Similarly, by using a high virulent strain M771 to induce infection, a group of orally immunized rats with attenuated ME49 appeared to not form muscle cysts [25].

Based on these findings, Falcon and Freyre (2009) attempted to use the same strain (ME49) to determine the possibility of using the rat model into sheep. A group of seronegative sheep received vaccination via oral inoculation of ME49 oocyst [23]. Then they challenged with a high pathogenic M3 strain which was isolated from aborted sheep in Scotland [22]. After a period lasting about 45 days post inoculation, the immunized lambs were euthanized and their meat samples were taken to feed mice which are highly susceptible to all Toxoplasmas' strains. No mortality was observed within the group of mice [23].

Although, the findings demonstrate that using of Toxoplasma strain (ME49) as a vaccine may provide a better immune response and prevent formation tissue cysts, it is not

apparent whether the vaccine could hinder brain cyst formation or prevents abortion in sheep.

*B. DNA Based Vaccines*

The majority of recent investigations have been focused on using plasmid DNA encoding for various antigens that are secreted by the *T.gondii* because it offers certain advantages; First of all, it has the ability to elicit both cellular and humoral immune responses, both with MHC I confined to cytotoxic T Cells and MHC II helper T cells. Moreover, only a few amount of vaccine is sufficient for inducing immune responses [26,27]. Furthermore, the Plasmid DNA has shown to provide protection against intracellular parasites such as Plasmodium [28] and Lishmania spp. [29].

Most of those investigations have been conducted using different proteins secreted by *T.gondii*, including micronemial protein (MIC3) and dense granule proteins (GRA1, GRA4, GRA6 and GRA7) [30]. Selection of these proteins based on their expression during different stages of toxoplasmosis such as tachyzoite and bradyzoite as well as their ability to induce strong antibody response during infection [31].

**1. Microneme (MIC) Protein as a vaccine candidate**

One of the most studied vaccines is a DNA that encodes for miconeme (MIC3) gene. Generally, the term microneme (MIC) covers proteins that aggregate at the apical surface of the Toxoplasma parasite, examples are MIC3, MIC6 and MIC8. They are involved in the attachment and invasion of the parasite into the host cell, particularly during the initial phase of the infection [32,33].

Among various micronemial proteins, MIC3 has been extensively studied. Firstly, due to its ability to express at various stages of the parasite (tachyzoite, bradyzoite and sporozoite), which could provide immunity at different stages [34]. Additionally, it can act as an immunogen through triggering specific T cell responses especially Th1. Furthermore, it contains epitopes that have the ability to elicit lymphocyte proliferation. Particularly, Peripheral Blood Monocyte Cells [35].

Performing intramuscular injection of a DNA vaccine containing a plasmid encoded for MIC3 protein, Ismael and coworkers observed a significant level of immunoglobulin production towards MIC3 in mice. Beside detection of IFN-γ and IL-2 which reveals both Th1/Cellular and IgG/Humoral immune responses, most predominantly IgG1and IgG2 [34].

A similar result was obtained in sheep by using the same vaccine comprising MIC3 gene with the only exception in applying a booster dose. However, the level of IFN-γ showed to be higher after first injection and immunoglobulin level shifted toward IgG2 after the booster dose. This finding could confirm the immunogenic characteristic of MIC3 in relation to triggering an appropriate immune mechanism against the *T.gondii*. Yet, it has not been found whether the vaccine has a sentinel activity to eliminate or prevent the formation of muscle or brain cyst which is of high zoonotic risk [36].

**2. Surface Antigens**

As survival attempts, both the host and the parasite require persistent balance between triggering and suppression of the host responses [37]. Surface antigens are responsible for interaction between the hosts and parasite. Therefore, they gained much attention by researches to determine their actual roles including SAG and SAG related sequences [13,37]. It is known that SAG1 and SAG3 which are found on the surfaces of

bradyzoite and tachyzoites play key roles in attachment and possibly invasion [38, 39].
Early attempts were focused on developing a major surface antigen such as SAG1 derived peptides [40], recombinant SAG1 in E.coli [41] or yeast [28] in a hope to find a proper immune trigger. These attempts brought insight to develop a DNA based vaccine encoding SAG1. The application of the later has shown to provide immune response in rodents both by reducing tissue cysts (Brain cyst) and decreasing mortality [42].

In the light of these findings, using sheep model, Li and his co-worker have tried a DNA encoding SAG1 containing ODN which also entailed oligonucleotide comprising CG motifs and the gene encoding GM-CSF (Granulocyte Monocyte- Colony Stimulating Factor) derived from different strains of *T.gondii*[43].

It has been observed that a significant level of protection can be obtained ranging from production of high level of antibodies, particularly, IgG1 and IgG2, in addition to very few brain cysts formation. However, the vaccine failed to enhance IFN- γ production which is quite crucial for elimination of the intracellularly localized *T.gondii* as a part of immune response to infection[43].

Possible reason for failure of IFN- γ production is that the strains in which the SAG1 obtained from might have contributed on the level of cytokine responses, as Rodgers *et al.* suggested that cytokine levels may be affected by *T.gondii* strains present during infection [44].

### 3. Dense Granule Antigens

Among various proteins present onthe *T.gondii* surface, there is a group of proteins dubbed Execretory-Secretory antigens in which they are expressed during both bradyzoites and tachyzoites stages, responsible for triggering a protective immune response during infection [38,45]. Dense Granule Antigens (GRAs) are among this group, several of these antigens have been identified including GRA1, 2, 4, and 7 [13], in particular, GRA1 and GRA7 which have been extensively researched as vaccine candidates in sheep.

For the GRA1, good results have been obtained; using a recombinant BCG strain of *Mycobacterium bovis*, Supply and coworkers revealed the effectiveness of GRA1 in sheep [46]. The BCG strain has adjuvant properties particularly for inducing cell mediated immune response [43]. This was successfully achieved in sheep through subcutaneous injection of recombinant BCG producing GRA1, with observing strong immune response, both with T cell and production of high level IFN-γ. Similar to S48 strain, the applied vaccine also resulted in generating some degrees of fever.

On the other hand, no specific antibodies for GRA1 were found, which is crucial for normal elimination of the parasite. Beside this, it has not been revealed whether the vaccine could avoid abortion or prevent the tissue cyst formation in sheep [46].

Following the above demonstrations, the scope of researches has paid particular attention to the GRA7. As a component of dense granule, GRA7 appeared to be released into the parasitisposous vacuole (PV) during natural infection, which then aggregates in the vacuolar space close to the membrane [47]. Transferring of the GRA7 from PV membrane into the cytoplasm of the target cell and its consequent uptake by adjacent cells directs the *T.gondi* to antigen processing through cystosolic and endosomal pathways. Hence, it provokes its recognition by T lymphocyte through MHC-I or MHC-II [48].

Using mouse model, the plasmid DNA encoding for GRA7 appeared to induce both cellular and humoral immune response characterized by synthesis of high level of IFN-γ which is crucially important for removing of the parasite [31]. However, due to the fact that application of DNA vaccines in large animals mainly depend on a couple of factors, involving route of administration, dose and expression level which may be not enough for inducing appropriate immune response. Hence, the findings in mice may not be directly and exactly applied in large animals [49].

In an attempt to ameliorate the effectiveness of vaccines against toxoplasmosis, different adjuvants have been evaluated in combination with plasmid DNA encoding GRA7 in sheep. Among those are liposomes which is found to be effective [27]. The reason of application of liposomes as adjuvant was based on its successful usage for gene delivery in humans. In addition, the plasmid enclosed in liposomes is not accessible to the nuclease enzyme. Hence, it prolongs the remaining of DNA for a longer period of time and a consequentlong lasting immunity [50].

The intramuscular injection of the above mentioned vaccine in sheep is found to results in production of a considerate to high level of both IFN-γ and IgGs. In particular, IgG1 and IgG2, indicating a potent immune response, more specifically toward Th1 cells. Additionally, it is also demonstrated that two sets of injections will provide the same result [27]. This could be an evidence of the feasibility of using GRA7 with liposome in obtaining a protective response within one injection.

## 4. Cocktail DNA Vaccine

In the light of different findings and based on the observation that different GRA proteins can induce a strong T cell response especially during chronic toxoplasmosis. It was assumed that a combination of these antigens together may result in obtaining a better immune protection [51,52]. This assumption has been successfully tested in mouse model by using a cocktail DNA containing both GRA1 and GRA7. The achieved results were made of both cellular and humoral immune responses along with lack of tissue cysts [31].

A relatively similar result have been obtained by using the same approach (cocktail of DNA) in sheep, in which different GRA proteins including GRA1, 4, 6 and 7 encoded in plasmid DNA injected intramuscularly to different groups of sheep. All of GRAs resulted in providing good immunity characterizing by production of antibodies and IFN-γ. Also it is worth noting that among a cocktail of DNA encoding for different GRAs, a specific immune response against GRA7 has been detected especially, specific IgG1 and IgG2 [49].

Although, the study only measured the immune response without exposing animals to the parasite, it confirms the previous investigation in mice in which GRA7 response emerged as a potential immune inducer among a cocktail of plasmid DNA encoded for various GRA proteins [31].

## 5. Rhoptries

Among Excreted-Secreted antigens (ESA), rhoptries are also proteins situated at the apical surface of the *T.gondii*. They are required and involved in the invasion and interaction within host cells [53,54]. Moreover, they have also been indicated as important virulence factors. This is mostly due to their involvement in the formation of parasitophorous vacuole (P.V.) which enables the parasite to multiply and resist intracellular killing. Therefore, they are qualified to be used as potential vaccine candidates [56].

The only trial in sheep has been carried out by Li and his coworkers on plasmid DNA encoding ROP1 in which different level of protections were achieved, ranging from INF-Y, IgG1 and IgG2 production [43]. Despite the fact that the vaccine was applied in combination with SAG1, it is the only study about the possibility of using ROP as vaccine in sheep.

Recently, a number of studies have been conducted in mice, using DNA vaccines encoding for different rhoptry proteins. This might even slightly go beyond the scope of this article, but it is worth pointing out that the results were promising, based on the fact that both cellular and humoral immune responses were gained, example of those proteins were ROP2 [57,58], ROP5 [59], Rop16 [60] and Rop18. The latter, in particular, has grabbed much attention due, presumably, to demonstrating a remarkable and strong antibody response in the trail; this was accompanied by production of cytokines such as INF-Y, IL-2.

Additionally, the vaccine has shown to prolong survival of the vaccinated mice, when they exposed to virulent strain RH, suggestion that using ROP18 as a vaccine can provide a long lasting immunity [61]. However, all of these findings were in mice, hence, confirmation through experimental studies in sheep are also required.

## III. Concluding remarks

In the light of our understanding, from the biological structure to the disease mechanism caused by the *T.gondii*, a couple of aspects should be considered during selecting a vaccine candidate. A potential vaccine in sheep should be able to elicit a strong and long lasting immune response, avoid transmission of the parasite to the fetus via placenta and provides protection during different stages of parasitic infection as well as prevents cyst formation either in muscle or brain, thus, by this way; it will avoid transmission to humans.

Based on the present published literatures on ROP proteins, the ROP18, which is explained as an important virulence factor of the*T.gondii* appears to be promising in developing a potential vaccine. Presumably, as single or in combination with other antigens, as the latter form has shown to be effective in several trials. In this regard, the feasibility of ROP18 as a vaccine candidate requires to be verified by further studies.

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