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# Apicomplexan profilins in vaccine development applied to bovine neosporosis

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## HIGHLIGHTS

- Apicomplexan profilins have emerged as promising vaccine and adjuvant candidates.
- Profilins tackle Toll-Like Receptors that may trigger protective responses.
- *N. caninum* profilin (NcPRO) induced regulatory T-cell responses in mice.
- In cattle, NcPRO only induced a primary immune response.
- Animal model is a critical issue for vaccine development against bovine reosporosis

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## ABSTRACT

*Neospora caninum*, an intracellular protozoan parasite from the *phylum Apicomplexa*, is the etiologic agent of neosporosis, a disease considered as a major cause of reproductive loss in cattle and neuromuscular disease in dogs. Bovine neosporosis has a great economic impact in both meat and dairy industries, related to abortion, premature culling and reduced milk yields. Although many efforts have been made to restrain bovine neosporosis, there are still no efficacious control methods. Many vaccine-development studies focused in the apicomplexan proteins involved in the adhesion and invasion of the host cell. Among these proteins, profilins have recently emerged as potential vaccine antigens or even adjuvant candidates for several diseases caused by apicomplexan parasites. Profilins bind Toll-like receptors 11 and 12 initiating MyD88 signaling, that triggers IL-12 and IFN-γ production, which may promote protection against infection. Here we summarized the state-of-the-art of novel vaccine development based on apicomplexan profilins applied as antigens or adjuvants, and delved into recent advances on *N. caninum* vaccines using profilin in the mouse model and in cattle.



## 1. Introduction

*Neospora caninum* is a worldwide distributed apicomplexan parasite, closely related to *Toxoplasma gondii*, which is considered one of the most relevant causes of reproductive failure in cattle, affecting both dairy and meet industries. It has an heteroxenous life cycle, in which canids are the definitive hosts (McAllister et al., 1998); and a wide range of domestic and wild animals (cattle, buffalos, horses, sheeps, cats, dogs, etc.) behave as intermediate hosts (Dubey and Schares, 2011). This parasite is the causative agent of bovine neosporosis, a disease that has a great economic impact mainly due to reproductive failure, premature culling and reduced milk yields (Dubey and Schares, 2011; Innes et al., 2005). Depending on the stage of the gestation in which the intra-utero infection occurs (Innes et al., 2002), bovine neosporosis can be associated to abortions due to inflammatory or degenerative fetal lesions; autolytic or mummified fetuses, birth of weak calves with nervous symptoms or the birth of clinically healthy, but chronically infected calves (Andrianarivo et al., 2001). Globally, the estimated median losses due to *N. caninum* induced abortions were valued in US $1298.3 million per annum (range US $633.4 million to US $2380.1 million), affecting both dairy and beef industries (Reichel et al., 2013). There are no vaccines or treatments commercially

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available against neosporosis and only management strategies are used (Wallach et al., 2008). In this context, the development of an efficacious vaccine is paramount.

Diverse vaccine-development strategies have been pursued to protect against diseases caused by apicomplexan parasites, most of them significant and harmful diseases affecting both humans and livestock (Wallach et al., 2008). Some of these vaccines reached the market. A live-attenuated *Eimeria* vaccine containing seven species of *Eimeria acervulina* oocysts delivered via the drinking water (Paracox®) was reported to protect against infection and disease (Shirley and Bedrník, 1997). Toxovax™, an effective live vaccine against toxoplasmosis in sheeps based on attenuated tachyzoites, is available in Europe and New Zealand (Verma and Khanna, 2013). However, production, storage and transport of live vaccines may be hard and expensive; besides the risk of reversion to virulence. For this reason, many efforts have been done during the last decades in order to replace these vaccines with effective inert antigens.

Apicomplexans are intracellular parasites, consequently, processes that allow cell invasion are critical and proteins involved therein represent potential targets for vaccination. Several experimental vaccine-developments entailed the use of proteins involved in the processes of adhesion and invasion of the host cell by sporozoites, tachyzoites and/or merozoites. Many studies were focused on *T. gondii*, which is considered the model of the *phylum*. Antigens were either native or recombinant proteins, administered as protein or as DNA vaccines. Most of these proteins are secreted by rhoptries, micronems and dense granules, structures restricted to the apical end of the parasite (*"apical complex"*). Different vaccines based on proteins from *T. gondii* used as recombinant vaccine antigens such as MIC1 (Ismael et al., 2003), MIC3 (Nie et al., 2011), MIC8 (Liu et al., 2010), ROP18 (Yuan et al., 2011) or SAG1 (Tang et al., 2016) induced partial protection against experimental infection in mice. Vaccination with parasite-derived or recombinant AMA1 from *Plasmodium falciparum*, when correctly folded, induced antibodies that inhibited parasite growth *in vitro* and protected mice and monkeys from blood-stage challenge (Remarque et al., 2008). Phase 1 studies have also been carried out in children with this formulation (Thera et al., 2010). ROP2, MIC1, MIC3 (Debache et al., 2009), GRA1, GRA2 (Ellis et al., 2008), and SAG1 (Cannas et al., 2003) from *N. caninum* have also been evaluated in different experimental mouse models.

Recognizing protozoan parasites is a complicated task for the mammalian immune system, mainly because the majority of the classical bacterial and viral molecules that are sensed by innate immune receptors are not found in eukaryotic pathogens (Takeuchi and Akira, 2010; Yarovinsky and Sher, 2006). Instead, the innate immune system seems to recognize them by recognizing a distinct set of molecules that are uniquely present in protozoan parasites (Plattner et al., 2008; Hou et al., 2011; Debierre-Grockiego et al., 2007). Among proteins involved in adhesion/invasion processes, profilins play an essential role, as they have also immunological function, activating the innate immune system of the host. These features have promoted profilins as promising vaccine antigens or adjuvant candidates. In this review we summarized the state-of-the-art of novel vaccine development based on apicomplexan profilins applied as recombinant antigens or adjuvants, and delved into recent advances on *N. caninum* vaccines using profilin in the mouse model and in cattle.

## 2. Apicomplexan profilins: the novel vaccine candidates

Apicomplexan parasites have no external organelles for locomotion. Instead of that, they have developed a unique form of substrate-dependent motility known as "*gliding motility*". This process is essential for the invasion of the host cell, in which proteins secreted by micronemes allow the adhesion to the host cell while proteins of the rhoptries begin the first steps of cell invasion, penetration of the parasite and the subsequent establishment within a structure known as parasitophorous vesicle (Bradley et al., 2005). In the inner membrane of the apical complex short actin filaments are responsible for the translocation of myosins and adhesins, allowing the motility of the parasite (Meissner et al., 2002; Loisel et al., 1999; Romero et al., 2004). Profilins are small monomeric actin binding proteins that play multiple roles in the regulation of the polymerization of those actin filaments. They were initially shown to sequester G-actin, thereby resulting in actin filament depolymerization (Carlsson et al., 1977).

Once the parasite enters the cell, profilin becomes dispensable as it is not involved in the replication processes or in the egress of the infected cell. In fact, *Toxoplasma gondii* profilin (TgPRO) is passively released from parasites through an unknown mechanism (Plattner et al., 2008; Yarovinsky et al., 2005). The innate immune system seems to recognize the presence of apicomplexan parasites by detecting the presence of this protein through Toll-like receptors (TLRs). These are receptors present in cells of the vertebrate's innate immune system, members of the pattern-recognition receptors (PRRs), which senses the presence of invading microorganisms triggering pro-inflammatory responses (Plattner et al., 2008; Hou et al., 2011). Thus, the immune system detects an early event associated with cell invasion and motility.

Profilins are important actors of the immune response against the parasite. By binding to TLR11 and TLR12, TgPRO activates murine dendritic cells and macrophages to release IL-12, which is involved in the production of IFN-γ via the MyD88-dependent pathway (Mineo et al., 2009) and in the differentiation of naïve T lymphocytes to Th1 phenotype (Plattner et al., 2008; Yarovinsky et al., 2005; Yarovinsky, 2014; Koblansky et al., 2013; Raetz et al., 2013; Lauw et al., 2005; LaRosa et al., 2008; Scanga et al., 2002). TgPRO also binds to TLR5 on human peripheral-blood mononuclear cells (PBMCs) (Salazar Gonzalez et al., 2014). Structural analyzes have been performed in order to describe the molecular basis of binding of TgPRO to TLR11, identifying two motifs, an acidic loop and a β-hairpin, that are highly conserved among different organisms of the *phylum* Apicomplexa (Kucera et al., 2010). Conditional disruption of the TgPRO gene prevented gliding motility and TLR11-dependent IL-12 production by the host's immune cells.

TgPRO was assessed as a vaccine candidate in mice (C57BL/c), formulated with liposomes covered with oligomannose as adjuvant. This experimental vaccine induced high titers of antibodies (mainly IgG2) and IFN-γ, conferring survival rates of 70% after challenge with live *T. gondii* tachyzoites (Tanaka et al., 2014). Recently, Neal and Knoll (2014) demonstrated that *T. gondii*-infected mice are more resistant to the bacterium *Listeria monocytogenes* due to the stimulation of murine TLR11 by TgPRO that can recruit inflammatory monocytes (Neal and Knoll, 2014). TgPRO has been recently used as a cancer-vaccine adjuvant in a murine model, promoting immune-cell migration, phagocytosis and activation of macrophages (Pyo et al., 2016) (Table 1).

Mouse splenocytes treated with *Cryptosporidium parvum* profilin (CpPRO) produced high levels of IL-12. When this protein was delivered in heterologous prime-boost schedules as *Salmonella* live vector vaccine and as purified recombinant antigen respectively, a specific and potent immune response (both humoral and cellular) was induced, suggesting its potential as a novel vaccine candidate against cryptosporidiosis (Manque et al., 2011) (Table 1).

Chickens immunized subcutaneously with *Eimeria acervulina* profilin (EaPRO) and an adjuvant containing QuilA, cholesterol, dimethyldioctadecyl ammonium bromide and Carbapol (QCDC) showed greater weight gain and reduced intestinal lesions after experimental infection by *E. acervulina*. Compared to control group