Chapter 9

Vaccination against *Trichinella spiralis*: Potential, Limitations and Future Directions

Jonathan I. Andrade-Becerra,
Ericka N. Pompa-Mera,
Rosa María Ribas-Aparicio and Lilián Yépez-Mulia

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Abstract

Trichinellosis is a food-borne parasitic disease caused by round worms of the genus *Trichinella*. The majority of human outbreaks are attributed to consumption of raw or undercooked pork meat contaminated with *T. spiralis* muscle larvae. A blocking-transmission vaccine against trichinellosis will allow preventing swine infection and will contribute to disease control. In this chapter, different vaccine candidates so far developed against *T. spiralis*, including first-, second-, and third-generation vaccines, are discussed. Most vaccine candidates are based on a unique antigen mainly from the muscle larva stage, inducing with some exceptions, partial protection although a mix Th1/Th2 immune response is elicited. Therefore, the need for identification of new antigens from different parasite stages focusing on infective intestinal larvae, adult, and newborn larvae stages as well as the evaluation of their protective capacity in pigs is presented. The design of multi-epitope vaccines and the use of adjuvants or immunomodulatory molecules capable to polarize the immune response to a Th2-type-protective response are discussed as imperative elements of modern vaccines. Plant-based vaccines and probiotics as excellent tools for vaccine development against *T. spiralis* are also presented as an attractive platform for veterinary vaccines.

Keywords: *Trichinella spiralis*, DNA vaccine, live carriers, edible vaccines, probiotics

1. Introduction

Trichinellosis is a significant global zoonotic disease produced by the nematode species of the genus *Trichinella*. Trichinellosis is an emerging and reemerging disease in many countries [1]. In the international ranking of food-borne parasites, *T. spiralis* was ranked among the

top 10 [2]. *T. spiralis* is the best characterized member of *Trichinella* genus since it is highly infective for sylvatic and domestic animals as well as for humans. Besides, its life cycle can be maintained in experimental animals, providing information about host-parasite relationships and immunity. Infection with *T. spiralis* initiates when the host ingests raw or undercooked meat contaminated with encysted muscle larvae (ML) (**Figure 1**). The larvae are released from muscle tissue by host digestive enzymes in the stomach. Then, ML migrates to the small intestine where they penetrate the intestinal mucosa and undergo four successive molts, becoming mature adult worms. This intestinal phase is the first stage in the host-parasite interplay. At days 1 and 2 post infection, newborn larvae (NBL) are released by female adult worm and spread via the blood and lymphatic systems to striated muscle, where they invade the myofibers, develop into ML, and induce the transformation of infected cells to the nurse-cell complex.

T. spiralis continues to be the causative agent in most outbreaks in humans. The majority of outbreaks are attributed to domestic pork maintained in small farms or non-controlled outdoor backyard pigs, where poor husbandry conditions place pigs at high risk. From 1986 to 2009, there were 65,818 cases and 42 deaths reported from 41 countries, 50% of those occurred in Romania, mainly during 1990–1999 [3]. In China, from 2005 to 2009, 15 outbreaks of human trichinellosis with 1387 cases and four deaths were recorded in three provinces of southwestern China. Twelve of these 15 outbreaks were caused by the eating of raw or undercooked pork meat [4]. The animal health situation varies between different countries being Argentina and some Eastern European countries where most of the cases were reported in pigs in 2015 [5].

T. spiralis infection induces a complex host immune response against a diversity of stage-specific antigens. Up to now, it is well known that during the intestinal phase of infection, the immune response involves a Th1/Th2 response with predominance of the Th2 phenotype characterized by the production of high levels of cytokines IL-4, IL-5, IL-9, and IL-10 as well as IgE, IgG1, and the mobilization of eosinophils and mast cells. Furthermore, the long-lasting

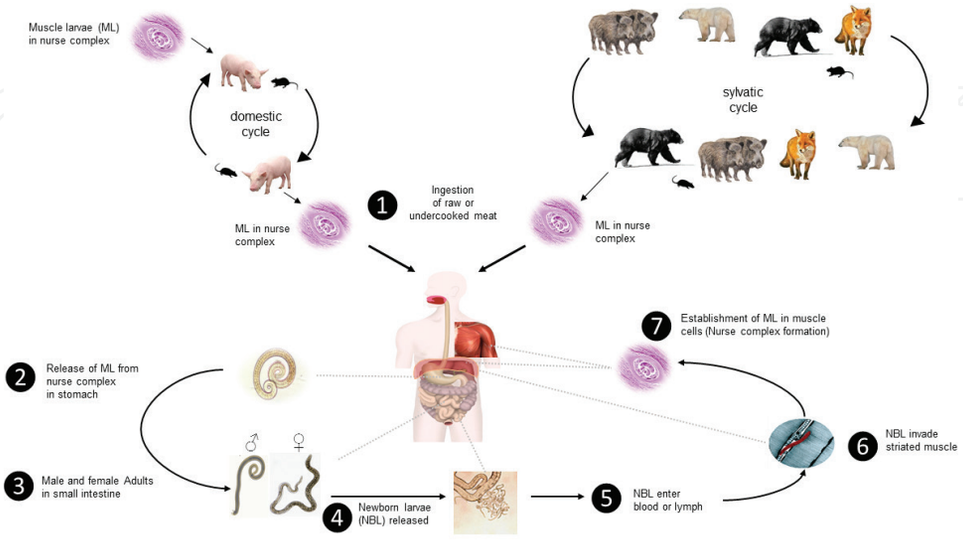

Figure 1. *Trichinella spiralis* life cycle.

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infection of muscles with *Trichinella* reflects successful immunomodulation of the immune response, mainly characterized by a Th2 phenotype [6].

Despite the availability of effective and relatively safe drugs such as albendazole and mebendazole for trichinellosis treatment, chemotherapy has several disadvantages such as treatment failure, parasite drug resistance, poor drug absorption in the intestinal lumen, and low bioavailability. Besides, traditional anthelminthic drugs are active against enteric stages of *T. spiralis*, but currently no anthelminthic drug has proven to be effective against the parasite systemic stages [7, 8]. Furthermore, serious side effects including bone marrow suppression and teratogenic effects are observed [7, 8].

An alternative for trichinellosis control is vaccination of livestock. Indeed, veterinary vaccines have already made enormous impacts not only on animal health, welfare, and production but also on human health [9]. Vaccines have been demonstrated to be efficient, reliable, and sustainable method to control parasitic infections, and have been referred as a green solution [10].

The aim of this chapter is to update the advances so far achieved in the development of a transmission-blocking vaccine against trichinellosis to prevent swine infection. Trichinellosis vaccine would make a practical contribution to disease control, reducing the production of residues in meat and food chain, eliminating the risk for the consumer and in some cases to improve the productivity of the individual animal.

The first part of the review presents an overview of *T. spiralis* antigenic molecules proposed as first- and second-generation vaccines, discussing the need for identifying and characterizing antigenic molecules from NBL and adult worm, mainly recognized by *T. spiralis*-infected swine, administered with adjuvants or delivered by carrier systems. The second part provides a description of third-generation vaccines (DNA vaccines) delivered as naked DNA or by carrier systems. Some experimental data recently obtained by our research group using second- and third-generation vaccines will be presented. Finally, the alternative use of adjuvants, multi-epitope vaccines, plants as a system to express antigenic molecules, and probiotics to protect against parasite infection will be discussed too.

2. First- and second-generation vaccines against trichinellosis

The biggest challenge for vaccine development is the identification of the best *T. spiralis* antigens that elicit host-protective immunity in terms of safety and protection at the both enteral and systemic levels. Different antigenic preparations from different parasite stages using different adjuvants have been tested as vaccine candidates. Most information related to immunity elicited by vaccine candidates have been mainly obtained from rodent models and only few studies have been performed in pigs.

2.1. First-generation vaccines

First-generation vaccines developed against trichinellosis include the use of autoclaved *T. spiralis* larvae and inactivated ML administered with complete Freund's adjuvant (CFA). These types of

vaccines induced in immunized mice significant ML burden reduction, as well as degeneration and hyalinization of the nurse-cell structure, accompanied by early pericystic fibrosis [11, 12]. In addition, antigenic preparations from the different stages of *T. spiralis* have been used in protection assays in mice. In this way, adult total extracts and ML total extracts provided protection against adult (89–74%) and ML (80%) stages. Importantly, ML total extracts induced the reduction of female fecundity (74%). The combination of adult and ML total extracts reduced the adult and ML load by 96% and 86%, respectively, and 73% reduction in female fecundity [13]. Protection assays performed in pigs have explored the use of excretory/secretory (E/S) antigens from *T. spiralis* ML and NBL total extracts [14, 15]. E/S products administered with CFA or aluminum hydroxide induced moderate protection mainly directed against the fecundity of female worms [14]. On the other hand, NBL killed by freezing and thawing combined with CFA were highly protective in swine (78%) against *T. spiralis* challenge, compared to 40% protection elicited with E/S products of ML [15]. These assays established that in pigs the immune response is mainly directed against fecundity of female worms and to the NBL.

It is worth mentioning that most of the studies have focused on ML antigens. Indeed, ML antigens are released and presented to the host immune system twice: by ingested ML in the intestine, and again when the new generation of ML becomes resident in muscle cells. Besides, ML antigens play an important role in the invasion of intestinal epithelium and therefore in the establishment of the infection in muscle cells. Even more, *T. spiralis* ML surface and E/S antigens are recognized by a wide range of hosts [16]. The carbohydrate epitope tyvelose confers the immunodominance to surface and E/S ML antigens [17]. Anti-tyvelose antibodies inhibit parasite invasion of an *in vitro* model of epithelial cells [18, 19]; however, tyvelose failed to elicit in mice a protective immune response against the enteral phase of infection [20].

Because of their antigenicity, the protection induced by ML surface and E/S products was extensively evaluated in mice [21–23]. In all these assays, partial protection against *T. spiralis* challenge was obtained as assessed by the reduction of adult and ML burden as well as female worm fecundity (35–58%).

A further step was achieved with surface and E/S stage-specific antigens purified by specific monoclonal antibodies [21, 24]. These antigens administered with CFA protected mice against parasite challenge, as determined by ML load reduction (29.6 and 50%, respectively). Protection induced by purified E/S products (49 and 55 kDa) was similar to that achieved when total E/S products were used [21].

Other E/S products, mainly glycoprotein of Mr 53 kDa (gp53) and 43 kDa (gp43), were widely investigated as first-generation vaccines. The role of these glycoproteins as mediators of intestinal epithelial cell invasion and niche establishment of *T. spiralis* has been suggested [18]. Even more, it was shown that antibodies against these glycoproteins inhibit *in the vitro* invasion of intestinal epithelial by *T. spiralis* [19]. Therefore, gp43 and gp53 are considered good vaccine candidates.

2.2. Second-generation vaccines

The 40- and 30-mer peptides derived from gp43 were synthesized and tested in protection assays [25, 26], giving rise to the development of second-generation vaccines against

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trichinellosis. The 40- and 30-mer synthetic peptides were administered to mice by intranasal (i.n.) or subcutaneous route with adjuvants such as the subunit B of cholera toxin (CTB) or incomplete Freund's adjuvant (IFA). The 40- and 30-mer synthetic peptides induced a significant reduction of adult worm burden against *T. spiralis* infection in comparison to control (36 and 64%, respectively). The immune response was characterized by the production of IgG1. Although the use of the synthetic peptides represents an innovative strategy for vaccine development, protection induced was not higher than that elicited with crude total extracts.

The induction of mucosal immunity plays an important aspect to be considered in the design of a blocking-transmission vaccine in which the use of liposomes, viral particles, and bacterial carriers has been used to deliver the selected antigen [27–31].

In this regard, *Salmonella*-based vaccine systems are considered among the most advanced and promising technologies developed to induce immunological protection against enteric pathogens because of their ability to both colonize the small intestine and invade non-phagocytic epithelial cells, thus allowing access to the underlying lymphoid tissue [32]. Taking advance of the use of *Salmonella* as live bacterial carrier, our group developed a *Salmonella* vaccine candidate expressing the 30-mer peptide derived from gp43 (amino acid residues 210–239, designated as Ag30) from *T. spiralis* ML. The autotransporter ShdA was employed to translocate Ag30 peptide to the surface of *S. enterica* serovar Typhimurium SL3261 [27]. Mice immuzed by i.n. route with the recombinant *Salmonella* pAg30 elicited a protective immune response against *T. spiralis* challenge, with 61.83% reduction of the adult burden and production of antigen-specific IgG1 and IL-5 (**Figure 2**). The use of the autotransporter MisL has also been used to translocate Ag30 to the surface of *S. enterica* serovar Typhimurium SL3261. The immunization of mice with the recombinant vaccine (i.n. route) in combination with an intraperitoneal (i.p.) boost with the recombinant protein induced a higher level of protection (76%) against the enteral phase of *T. spiralis* infection [28]. In addition, our group explore the use of the 40-mer peptide of *T. spiralis* gp43 protein (named Ag40) expressed on the surface of *S. enterica* serovar Typhimurium SL3261 using the autotransporter ShdA (*Salmonella* pAg40). Partial protection against *T. spiralis* infection at the enteral level was induced (47%). The use of *Salmonella* pAg30 together with *Salmonella* pAg40 did not elicit higher protection against *T. spiralis* infection (58%) [33].

To enhance the humoral and cellular antigen-specific immune response against *T. spiralis* infection, multiple copies of the minimum binding domain of complement C3 component (P28) were used as molecular adjuvant. For this, *Salmonella* pAg30 vaccine was engineered to express the Ag30 peptide from *T. spiralis* fused to three copies of P28 adjuvant (Ag30-$P28_3$) and was either expressed on the bacterial surface or secreted to the milieu [31]. *Salmonella* vaccines were administered to mice by i.n. route. Data showed that *Salmonella* strains secreting Ag30-$P28_3$ or Ag30 reduced the adult worm burden by 92.8 and 72%, respectively, following the challenge with *T. spiralis* ML compared to 42% achieved by recombinant *Salmonella* displaying Ag30-$P28_3$ on the surface (**Figure 2**). The protection induced by secreted Ag30-$P28_3$ was associated with a mixed Th1/Th2 with predominance of Th2 phenotype, characterized by the production of IgG1, intestinal IgA antibodies, and IL-5 secretion.

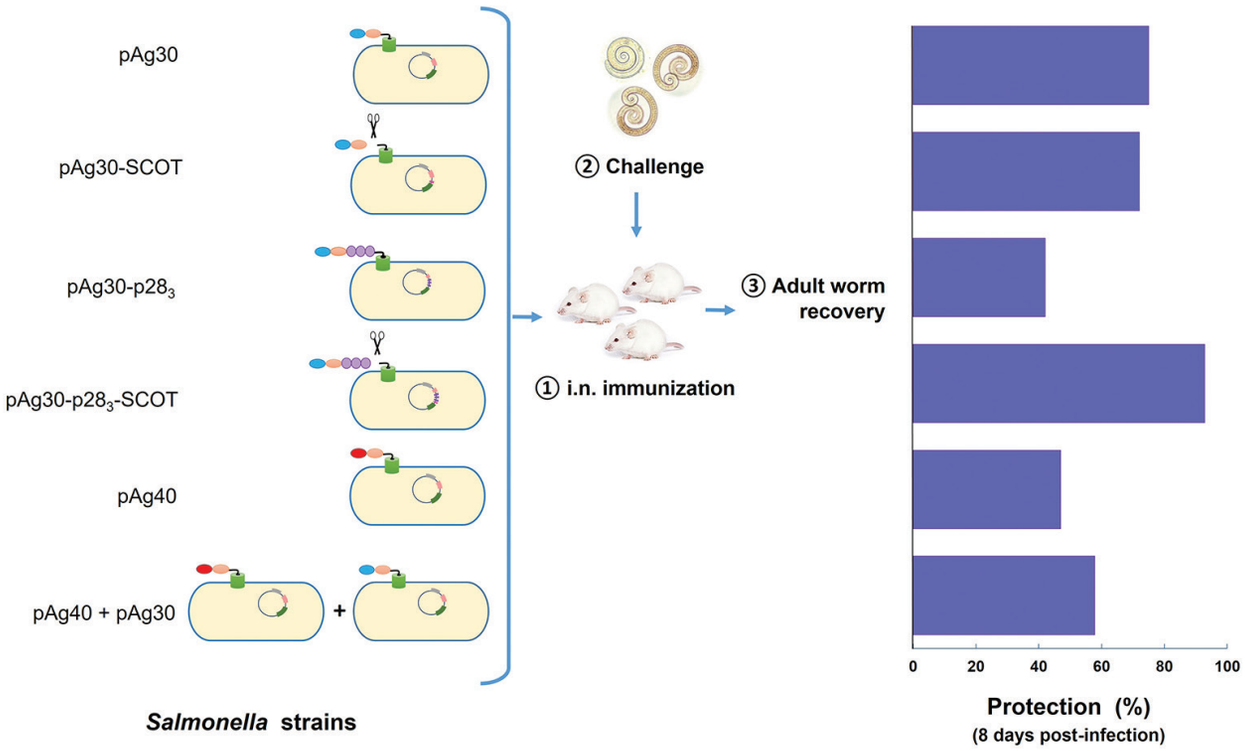

Figure 2. Protection in mice induced by recombinant *Salmonella enterica* serovar Typhimurium strains against the challenge with *Trichinella spiralis* muscle larvae. Attenuated *Salmonella* expresses Ag30 or Ag40 derived from the gp43 of *T. spiralis* muscle larvae displayed on the surface of recombinant *Salmonella* strains. In addition, Ag30 was fused to three copies of the molecular adjuvant P28 (Ag30-P28$_3$) and it was expressed on the bacterial surface or secreted to the medium through OmpT protease site (SCOT).

Some new surface and E/S proteins from ML (some of them expressed in other stages of the parasite) have been identified and their recombinant proteins evaluated as vaccine candidates. The surface protein 28.9 kDa (Ts14-3-3) [34], the E/S protein of 35.5 kDa (TspSP1.2) [35], 54.7 kDa amino peptidase (TsAP) also expressed by adult worms and NBL, located primarily at the cuticle and internal organs of the parasite [36], the protein of 20 kDa (Ts-ES-1) existing in the E/S products of *T. spiralis* adult and ML [37], and paramyosin (Ts-Pmy) [38] among others have been tested in mice. In all cases, partial protection assessed against the enteral and muscle phase of the infection was elicited (35–55%). Interestingly, a multiple epitope vaccine was developed including a highly antigenic epitope of Ts-Pmy (8F7) and an epitope (M7) from Ts-87 antigen (present in adult worm). Epitopes were conjugated to KLH and mixed to formulate a multi-epitope vaccine. This vaccine induced partial protection (35%) against *T. spiralis* infection in mice [30]. Protection elicited was not higher than that obtained with 8F7 or M7 alone.

Importantly, it was shown that ML cannot invade the intestinal epithelial cells *in vitro* cultured unless they are exposed to the intestinal milieu or bile and activated into the intestinal infective larvae (IIL) [39–41]. The identification of IIL molecules provides attractive information not only to elucidate the mechanism of parasite invasion and immune evasion but also to

identify possible molecular targets for vaccine. Following this purpose, several IIL molecules have been identified and their protective capacity evaluated. The Tsp10 polypeptide of *T. spiralis* IIL displayed on the surface of T7 phage was injected i.p. and intradermally at different sites of the abdomen, and elicited in immunized mice a Th2-protective response against parasite challenge, reducing the adult and ML load by 62.8 and 78.6%, respectively [29].

Other proteins with significant higher expression in IIL than in ML such as a putative copper/zinc superoxide dismutase (SODC), adult-specific DNase II, putative low-density lipoprotein receptor domain class A (LDLRA), and secreting receptor (SR) have been identified [41]. More recently, some important proteins were identified in E/S from IIL, such as the gp53 kDa with serine protease activity, multi-cystatin-like domain and cystatin-like protein, deoxyribonuclease II family protein, among others [42]. The protective evaluation of recombinant cystatin-like protein from *T. spiralis* IIL administered in mice with CFA and boosted with recombinant protein with IFA showed 62 and 64% reduction in the number of ML and adult worm, respectively. Interestingly, it was recognized by pig antiserum as early as 15 days post infection [43].

T. spiralis protein Nudix hydrolase (TsNd) is an up-regulated gene in IIL compared to ML. Recombinant TsNd emulsified with CFA displayed in mice a 57.7 and 56.9% reduction in adult worms and in ML burden, respectively, after a challenge infection with *T. spiralis* with high IgG1 levels [44].

Although rodent models have provided important knowledge about the immune response elicited against *T. spiralis* and immunogenic molecules recognized by sera from infected animals, it is important to mention two important aspects of trichinellosis that should be taken into account for the development of a vaccine. First, domestic pork consumption still accounts for many trichinellosis outbreaks, mostly in Eastern Europe and Argentina, where backyard pigs are raised under high-risk-rearing practices, especially the feeding of food waste. Second, a small number of studies have characterized adult antigens that stimulate immunity during an early infection and could be effective in host protection. In this way, recent studies have identified proteins from adult and ML that are recognized by sera of pigs experimentally infected with *T. spiralis* [45, 46]. Some proteins common from adult and ML stage have been identified, among them heat-shock proteins (HSPs), enolase, and 5'-nucleotidase. It was shown that HSP70 and a 38 kDa protein (Ts87) that is present in E/S products and on the adult cuticle induced protective immunity in mice assessed by ML burden reduction (38.4 and 29%, respectively) [46, 47].

It is worth noting that another important aspect that has to be considered is the anti-fecundity effects and immunity to the NBL described in *T. spiralis*-infected pigs [48]. Therefore, the identification of immunogenic proteins characteristic of NBL is important for the induction of protection and vaccine development that could be applied in swine. In this regard, an immunodominant serine protease, named NBL1, has been identified in NBL, embryos, and larvae before birth within the gravid females [49]. Importantly, sera from pigs experimentally infected with *Trichinella* and pigs immunized with the recombinant C terminal part of NBL1 allowed the recognition and identification of six immunodominant linear epitopes on

the protein [50]. These epitopes could be used for the development of subunit and multiple epitope vaccines.

2.3. Third-generation vaccines

DNA vaccines allow the *in vivo* expression of antigens in their native conformation, persistent expression of the desired antigen, and the induction of both humoral and cellular immunity [51]. Up to date, three DNA vaccines for veterinary use have been licensured (against West Nile equine virus, melanoma in dogs, and hematopoietic necrosis virus in salmon) [52], encouraging the improvement of experimental DNA vaccines against trichinellosis.

Several DNA vaccines using the eukaryotic expression vector pcDNA3.1 have been designed against *T. spiralis* infection and the induced immune response in mice evaluated. The 31 kDa E/S antigen of ML (TspE1) and TsNd, an up-regulated gene in IIL compared to ML, have been cloned in pcDNA3.1 vector [53, 54]. Recombinant pcDNA3.1-TsNd vaccine conferred higher levels of protection against *T. spiralis* infection in comparison to pcDNA3.1-TsE1. Vaccination of mice with pcDNA3.1-TsNd showed 40.44% reduction in worm adults and 53.9% reduction in ML burden with the production of a mixed Th1/Th2 systemic immune response and IgA production at the mucosal level [54]. In this case, the use of pcDNA3.1/TsNd did not increase the protection previously conferred by recombinant TsNd (57.7 and 56.9% reduction in adult and ML burden, respectively) [44].

The eukaryotic expression vector pVAX1 has also been used to express different *T. spiralis* antigens such as macrophage migration inhibitory factor (MIF) of *T. spiralis* (TsMIF), the protein domain of multi-cystatin-type 1 (MCD-1) (TsMCD-1), and the co-expression of TsMIF and TsMCD-1. Vaccination of mice with the recombinant vaccines induced low levels of protection (23.17% reduction of ML load) [55]. Slightly higher protection was achieved when pVAX1-ubiquitin vaccine was used (37.95%) [56]. In addition, the recombinant vaccines pVAX1-Ts87 and pVAX1-TsPmy conferred 9.7 and 46.6% protection against parasite challenge [57, 58]. Higher levels of protection (43.8%) were obtained when animals were co-immunized with pVAX1-Ts87 and recombinant Ts87. In both cases, a Th1/Th2 immune response was induced [57].

To avoid degradation of DNA vaccines by nucleases, the use of live carriers has been investigated. In this way, pVAX1-Ts87 and pcDNA3.1/TsNd were delivered by *S.* typhimurium strains SL7207 and SL1344, respectively [59, 60]. Mice immunized with *Salmonella* pcDNA3.1/TsNd showed higher levels of protection assessed by adult (73.32%) and ML (49.5%) load reduction [60]. In this case, higher protection at the enteral level was achieved than with the use of the DNA vaccine alone (73.32 vs. 40.44%).

2.3.1. DNA vaccine encoding Ag30

Since DNA vaccines have several advantages over protein vaccines, our research group developed a DNA vaccine encoding Ag30 using the pVAX1 vector (pVAX1-Ag30). The intramuscular administration of 50-μg pVAX1-Ag30 induced 54% reduction of adult burden in mice.

The use of *Salmonella* to deliver pVAX1-Ag30 failed to elicit higher protection levels at the intestinal level (22%) (data not published).

The use of liposomes as carriers of plasmid DNA has been used for vaccination purposes in various studies, because they act as adjuvants and protect plasmids from the attack of host enzymes [61]. It was our interest to assess the protection elicited by lipoplexes formed with 3-μg pVAXAg30 and cationic liposomes (LLO-LLE plus cholesterol, L-lysyl-octadecanol, and L-lysyl eicosanol). The intranasal administration of the lipoplexes induced in mice very low levels of protection against *T. spiralis* infection (7 and 9% reduction of adult and ML burden) (data not published).

Second generation	**Protection %**	**Reference**
Recombinant TsPmy	ML 36.2	[38]
Recombinant TsNd	A 57.7 ML 56.9	[44]
Recombinant Ts87	ML 39.7	[57]
30-mer synthetic peptide (Ag30)	A 36	[26]
Salmonella pAg30	A 61.8	[27]
Salmonella pAg30 (secreted)	A 72	[31]
Salmonella pAg30-p28$_3$ (secreted)	A 92.8	[31]
Salmonella pAg30-p28$_3$ (surface)	A 42	[31]
Phage T7-Tsp10	A 62.8 ML 78.6	[29]
Multi-epitope (Tspmy, TS87)	ML 35	[30]
Third generation		
pcDNA3.1-TsNd	A 40.44 ML 53.9	[54]
pVAX1-Ts87	ML 9.7	[57]
pVAX1-Ag30	A 54	Personal communication
Third generation + carrier		
Salmonella pVAX1-TsPmy	A 44.8 ML 46.6	[58]
Salmonella pcDNA3.1-TsNd	A 73.32 ML 49.5	[60]
Salmonella pVAX1-Ts87	A 29.8 ML 34.2	[59]
Salmonella pVAX1-Ag30	A 22	Personal communication

A, adult; ML, muscle larvae.

Table 1. Protection in mice induced by some second- and third-generation vaccines administered alone or delivered by carriers.

2.3.2. Protection induced by some candidate antigens as second- and third-generation vaccines delivered alone or by live carriers

A summary of protection elicited in mice by some candidate antigens proposed as second- and third-generation vaccines is presented in **Table 1**. Antigen and delivery systems are critical elements that influence the protection level induced by the candidate vaccines. Some antigens such as Ts87 used as second-generation vaccine or as third-generation vaccine delivered by *Salmonella* elicit similar protection against *T. spiralis* infection. In the case of Ag30, it improves the protection induced against the enteral stage of *T. spiralis* when it is administered as second-generation vaccine delivered by attenuated *Salmonella*, particularly when it is fused to the molecular adjuvant P28 and secreted to the medium. For TsNd, no differences in protection are observed with second- and third-generation vaccines; however, it elicits higher protection against *T. spiralis* adult as third-generation vaccine delivered by *Salmonella*. On the other hand, Tsp10, an IIL antigen displayed on the surface of T7 phage, induced the highest protection against the systemic stage of *T. spiralis*; however, at the enteral level the protection was lower. An important aspect to mention is the administration route of these candidate vaccines that is correlated with the protection elicited against the parasite challenge. The second-generation vaccine, *Salmonella* pAg30-p28$_3$ (secreted) administered by i.n. route, afforded at the intestinal level the highest protection against *T. spiralis* challenge (92.8%), followed by *Salmonella* pAg30 displayed by MisL (second-generation vaccine) (76%), also administered by i.n. route and *Salmonella* pcDNA3.1-TsNd (third-generation vaccine) (73.2%) administered by oral route. On the other hand, Tsp10, an IIL antigen displayed by T7 phage, provided the highest protection against *T. spiralis* ML (78.6%); it was administered by i.p. and intradermal via at multiple sites of mice abdomen. Therefore, the administration route also plays an important aspect to be considered in the vaccine development.

3. Adjuvants

The induction of mucosal immunity plays an important aspect to be considered in the design of a vaccine against *T. spiralis* infection. Therefore, it is desirable that vaccine formulations contain adjuvants or immunomodulatory molecules capable to polarize the immune response to a Th2-type response. Indeed, adjuvants can influence the balance of the induced antibody and cell-mediated immunity so constitute an imperative element of modern vaccines [62].

An adjuvant with documented *in vitro* and *in vivo* Th2-skewing properties is the cholera toxin subunit B (CTB) [63]. CTB has been used as potent immunological adjuvant in the induction of protective Th2-type response by first- and second-generation vaccines against *T. spiralis*. More recently, the second-generation vaccine, gp53 of *T. spiralis* ML, contained into virus-like particles with the influenza matrix protein 1 (M1) as a core protein was administered to mice together with CTB, inducing protective immunity against the parasite challenge [64].

Aluminum hydroxide adjuvant (alum) has also been administered with first- and second-generation vaccines. When administered with E/S products from *T. spiralis* ML, although it has been documented in mice to induce Th2 responses, no production of IL-5 was detected [65].

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The use of the adjuvant water in oil emulsions Montanide® ISA70 (Seppic, France) has been recommended for administration with first-generation vaccines, since its administration together with ML total extracts induced high level of protection (84.5%) against *T. spiralis* infection [66].

In order to enhance immunity, cytokines genes such as IL-4 have been included into third-generation vaccines (DNA vaccines) and have demonstrated to evoke a Th2-type response [67]. Interestingly, porcine IL-4 has been successfully evaluated as an immunological adjuvant in a vaccine candidate against porcine reproductive and respiratory syndrome virus (PRRSV) [68]. The cytokine IL-33 plays an important role at the mucosal level, inducing expansion of a multipotent progenitor cell population with differentiation into macrophages, basophils, and mast cell populations that promote the development of Th2 cytokine responses [69]. Further studies are necessary to determine the potential of IL-4 and IL-33 as molecular adjuvants in the induction of mucosal-protective immunity against *T. spiralis*.

4. Perspectives and future directions

4.1. Multi-epitope or polyvalent vaccines against trichinellosis

T. spiralis has a complex life cycle; the immune response elicited by a vaccine based on a unique antigen may not be strong enough to combat the challenging infection, and therefore multi-epitope vaccines against *T. spiralis* have been proposed. In this regard, the combination of three selected epitopes from Ts-Pmy and Ts87 from *T. spiralis* adult elicited in immunized mice IgG and IgG1 production and higher protection (35%) against the parasite challenge in comparison to that induced by individual epitope peptides [47]. To accomplish higher protective immune responses against *T. spiralis*, it will be necessary to design a vaccine with multi-epitopes from different parasite stages focusing on NBL and adult stages.

4.2. Probiotics in protection against *T. spiralis* infection

It has been demonstrated that probiotics modulate the intestinal environment preventing enteric infections. The lactic acid bacteria *Lactobacillu*s is considered as probiotic; they are part of the commensal bacteria and contribute to the maintenance of immune homeostasis in the gut [70]. The protective role of *L. casei* against high infection dose of *T. spiralis* has been demonstrated in mice inoculated intraperitoneally with the bacteria as assessed by adult (76.7%) and ML (80.9%) load reduction, production of high IgA and IgG anti-*T. spiralis* antibody levels as well as IL-4 [71]. More recently, the protection conferred by different *Lactobacillus* strains, *L. casei*, *L. plantarum*, and *L. acidophilus*, against *T. spiralis* infection was analyzed. The highest protection was elicited by *L. plantarum*, against adult (69.02%) and ML (87.92%). Interestingly, the authors demonstrated an amelioration of inflammation and damage in the intestine of *T. spiralis*-infected mice inoculated with *L. plantarum* with respect to non-treated-infected animals [72]. So far, the use of probiotics is considered as a new tool for trichinellosis control.

4.3. Plant-based veterinary vaccine

Plant-based vaccines might be used as edible vaccines for sustainable prophylaxis against various important parasitic diseases, including trichinellosis. Recombinant proteins based in plants can be produced in nuclear-transformed plants, synthesized in the cytoplasm, and can be accu-

mulated in different subcellular organelles, or secreted, once an appropriate transit or signal peptides are used [73, 74]. Plants are considered an attractive platform for veterinary vaccines, due to low-cost production, sterile delivery, and cold storage/transportation at ambient temperature, compared to traditional attenuated vaccines, which present some inconvenience in terms of insufficient mass production, residual toxicity, means of transportation, and safety [75]. Antigens administered by oral route are subject to proteolysis in gastrointestinal tract, reducing their bioavailability, and therefore affecting the quality of immune response. Then, vaccine antigens can be protected by plant cell walls from further degradation in the digestive tract, enabling them to reach the gut-associated lymphoid tissue [73].

Many species of plants, including tobaccos, alfalfa, spinach, potatoes, rice, beans, maize, tomatoes, strawberries, and carrots, can be used in plant biotechnology for the expression and production of foreign proteins, remaining stable without the loss of activity for years at room temperature. Hence, plants could be suitable for direct consumption and useful for the development of animal vaccines [74]. In fact, edible vaccines produced in papaya and corn seed induced protection against porcine-cysticercosis (70–90%) and porcine-transmissible gastroenteritis virus (50%) [76, 77]. Even more, edible vaccines can include adjuvants as it was the case for As16-an antigen protective against the roundworm *Ascaris suum* fused with CTB in transgenic rice seeds, resulting in an antibody response [78].

Plant-based vaccines represent an excellent tool for mass prevention especially at the veterinary field; their use in vaccine development against *T. spiralis* remains to be explored.

5. Conclusions

Different vaccine candidates based on antigens from different stages of *T. spiralis*, used as recombinant proteins or as DNA vaccines, delivered alone or by live carriers have been proposed. Most of them with some exceptions have induced partial protection against the enteral and muscle phase of the infection. In these studies, a mixed Th1/Th2 immune response with predominance of a Th2 response has been elicited. Up to now, the second-generation vaccines, *Salmonella* pAg30-p28$_3$ (secreted) and T7-Tsp10, have afforded at the intestinal and systemic level, respectively, the highest protection against *T. spiralis* challenge. Protection elicited by the candidate vaccines is influenced by the candidate antigen, delivery system, and administration route. Importantly, search for more useful vaccine candidates that could elicit high protection against *T. spiralis* infection in pigs is required. These vaccines may include antigens from IIL, NBL, and from pre-adult and adult stages of infection, administered alone or as multi-epitope vaccine. The use of adjuvants or immunomodulatory molecules capable to polarize the immune response to a Th2 type should be taken into account in a way to improve the protection induced by candidate vaccines. On the other hand, plant-based vaccines represent an excellent tool that needs to be explored in vaccine development against *T. spiralis* with application at the veterinary field.

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Abbreviations

Ag30	30-mer peptide derived from gp43 from *T. spiralis* ML
Ag30-P28$_3$	Ag30 fused to three copies of P28 adjuvant
Ag40	40-mer peptide derived from gp43 from *T. spiralis* ML
CTB	Cholera toxin subunit B
CFA	Complete Freund's adjuvant
E/S	Excretion/secretion products
gp43	Glycoprotein with molecular weight of 53 kDa from *T. spiralis* ML
gp53	Glycoprotein with molecular weight of 43 kDa from *T. spiralis* ML
HSP70	Heat-shock protein 70
IFA	Incomplete Freund's adjuvant
IIL	Intestinal infective larvae
i.n.	Intranasal
i.p.	Intraperitoneal
KLH	Keyhole limpet hemocyanin
ML	Muscle larvae
NBL	Newborn larvae
P28	Minimum binding domain of complement C3 component
TsAP	54.7 kDa amino peptidase
Ts-ES-1	Protein of 20 kDa existing in the E/S products of *T. spiralis* adult and ML
Ts14-3-3	Surface protein 28.9 kDa
TsMCD-1	Protein domain of multi-cystatin-type 1 of *T. spiralis*
Ts-Pmy	Paramyosin
Ts87	38 kDa protein that is present in E/S products and on the adult cuticle
TsMIF	Macrophage migration inhibitory factor of *T. spiralis*
TsNd	*Trichinella spiralis* Nudix hydrolase
TspE1	31 kDa E/S antigen of ML
TspSP1.2	E/S protein of 35.5 kDa

Author details

Jonathan I. Andrade-Becerra[1,2], Ericka N. Pompa-Mera[2], Rosa María Ribas-Aparicio[1] and Lilián Yépez-Mulia[2]*

*Address all correspondence to: lilianyepez@yahoo.com

1 Laboratory of Production and Biological Control, Department of Microbiology, National School of Biological Sciences, National Polytechnic Institute, Mexico City, Mexico

2 Investigation Unit in Infectious and Parasitic Diseases, Pediatric Hospital, Mexican Institute of Social Security, Mexico City, Mexico

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