Toward the Optimization of a Plant-Based Oral Vaccine Against Cysticercosis

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Abstract It is recognized that an effective, low-cost oral vaccine may effectively contribute to prevent *Taenia solium* cysticercosis; plant-based vaccines, on the other hand, can make this goal feasible. Plants are optimal platforms for the massive production of oral vaccines. In this chapter, advances toward the development of oral plant-based vaccine against cysticercosis are reviewed.

Keywords Oral vaccine · Transgenic plant · Transplastomic plant
Carica papaya · *Daucus carota* · *Nicotiana tabacum* · *Taenia solium*

1 Introduction

1.1 Relevance of Cysticercosis

Taeniasis/cysticercosis is a parasitic infection caused by *Taenia solium.* It is prevalent in areas with low socioeconomic and sanitary standards in developing

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countries. *T. solium* cysticercosis is still endemic in most countries of Asia, Africa, and Latin America despite significant progress in developing effective tools for its prevention, diagnosis, and treatment (Fleury et al. 2012, 2013).

T. solium cysticercosis can be acquired by humans (the definitive host) and pigs (the intermediate host) after ingesting parasite eggs in food or water contaminated with feces from human *T. solium* tapeworm carriers (Sciutto et al. 2000). A single tapeworm carrier may produce thousands of *T. solium* eggs per day, which are shed to the environment, contaminating vegetables, running waters, and soils, upon open-air defecation (De Aluja 2008). Humans also acquire intestinal tapeworms by eating insufficiently cooked meat from cysticercus-infected pigs.

In humans, cysticerci frequently establishes in the central nervous system (CNS), causing neurocysticercosis (NC), the most severe form of the disease. NC is a clinically and radiologically heterogeneous disease, ranging from an asymptomatic infection to a severe and eventually fatal disease. NC severity mainly depends on the location of the parasite. The most severe clinical forms occur when parasites are located in the subarachnoid space at the base of the brain. This form of the disease is also less susceptible to cysticidal drugs and more difficult to diagnose based on neuroradiological studies (Marcin Sierra et al. 2017).

Pig vaccination may curtail *T. solium* transmission by reducing the number of cysticerci, and thence the incidence of adult intestinal tapeworms in humans. Although several vaccines have been developed and successfully field-trial tested, no cysticercosis vaccines for pigs have been approved for commercialization. All of them induced high level of protection, but in all cases they are injectable vaccines (Huerta et al. 2001; Morales et al. 2008; Assana et al. 2010). Injectable vaccines are costly and their administration at a massive scale implies logistic difficulties, since pigs roaming in rural areas must be captured by trained personal for vaccination (Huerta et al. 2001; Morales et al. 2008; Assana et al. 2010). These difficulties limit the use of parenteral vaccines in nationwide programs and may underlie the lack of interest in companies to produce the vaccine commercially. An orally administered vaccine, which could be delivered by pig owners, would elude these difficulties.

1.2 Context of Veterinary Vaccines

Veterinary vaccines are aimed to reduce morbidity in animals for human consumption (chickens, cows, fish, and pigs), pets, and in wildlife species, to prevent the loss or contamination of animal derivatives (improve productivity), and to reduce the risk of disease transmission from animals to humans (zoonosis) (Meeusen et al. 2007).

According to the World Organization for Animal Health (OIE), 116 animal infections are included in the 2017 list of diseases, infections, and infestations. Most of these diseases are caused by bacteria such as *Salmonella* spp., *Listeria monocytogenes,* and *Escherichia coli;* viruses such as avian influenza virus, norovirus,

and rabies virus, as well as parasites such as *Taenia saginata*, *T. solium*, *Toxoplasma gondii,* and *Giardia duodenalis* (www.oie.int/es).

Several conventional commercial pig vaccines are currently being used to prevent infections in pigs caused by pathogenic *E. coli*, *Salmonella*, and *Lawsonia intracellularis* strains (Table 1) (Meeusen et al. 2007).

While most veterinary vaccines have been formulated for parenteral administration, the number of oral vaccines has increased in recent years, considering the advantages of this alternative route. Oral administration of vaccines can be performed by animal owners themselves. In addition, oral administration is less-costly than the parenteral route since neither trained personal for administration nor the use of needles are required. Moreover, the oral route is non-invasive and particularly attractive for the prevention of orally acquired infections like cysticercosis (Wang and Coppel 2008). Oral vaccines efficiently stimulate the mucosal system, improving protection by emulating the entry path of most pathogens. In addition, oral immunization can induce a concomitant systemic immune response (Muir et al. 2000; Mutoloki et al. 2015).

The mentioned advantages are especially relevant when designing an economic vaccine to prevent these neglected tropical diseases (NTDs) (WHO 2010) that affect rural pigs in marginalized populations of poor countries. In this context, an oral recombinant vaccine could overcome these limitations.

Several avirulent and live-attenuated oral vaccines are applied to pigs to prevent diseases like salmonellosis and erysipeloid. As in the case of poultry, a lyophilizate can be mixed with sterile water and administered as a beverage to 3–8 weeks-old pigs.

On the other hand, subunit vaccines based in immunoprotective proteins provide a safer approach for vaccination. An example is the rabies vaccine known as Raboral, in which glycoprotein G is used; it is administered as bait to wild animals in France, Belgium, Germany, and the USA (Bano et al. 2017).

Table 1 Orally-administered, commercial pig vaccines against bacterial pathogens

Pathogen	Disease	Brand name	Distributor	Composition	References
Lawsonia intracellularis	Porcine proliferative enteropathy	Enterisol® Ileitis	Boehringer Ingelheim Vetmedica, Inc.	LAT	Park et al. (2013)
Salmonella choleraesuis and *typhimurium*	Salmonellosis	Enterisol® Salmonella T/C		LAV	Wales and Davies (2017)
Salmonella choleraesuis		Enterisol® SC-54		LAV	
Erysipelothrix rhusiopathiae	Erysipelas	Ingelvac® ERY-ALC		LAV	http://www.bivetmedica.com/
Escherichia coli strain K-88	Enteritis	Entero Vac	Arko labs	LAV	Cox et al. (2014)

LAT live attenuated; *LAV* live avirulent

In addition to enhanced safety, an advantage of subunit vaccines is their ability to address multiple genetic variants of a pathogen in a single chimeric protein. Several pathogens, such as RNA viruses, exhibit a high mutation rate, resulting in a great variability within a short period; in addition, multiple serotypes are reported for several virus strains. Since many existing viral vaccines cannot recognize new viral strains, novel strategies to produce vaccines against these new infectious variants are much needed (Meeusen et al. 2007). In the case of parasitic diseases, an immune response against several antigens is desired to achieve an efficient immunoprotection. Subunit vaccines constitute an alternative to address these challenges, since the development of multivalent vaccines based in mixtures of several antigen variants or even in multiepitopic proteins carrying a set of relevant epitopes can provide broad immune responses.

1.3 Advances in the Development of Plant-Based Oral Vaccines

The expression of recombinant vaccine antigens in plants to elicit and maintain protective immunity is an attractive option that has been explored for the last two decades. Edible vaccines currently under development are based on fruits, leaves, and seeds of transgenic plants. Such vaccines are prepared without expensive antigen purification steps, commonly required for parenterally administered vaccines (Lugade et al. 2010) (Table 2).

Besides protecting against pathogen viruses and bacteria infecting domestic animal species, this strategy is appropriate to delivery parasite-derived antigens to gut-associated lymphoid tissues (e.g. for fasciolosis, schistosomiasis, coccidiosis, cysticercosis, and ascariasis) (Walmsley and Arntzen 2000; Chambers et al. 2016).

1.4 Transgenic Plants as Alternative Platforms to Produce an Anti-cysticercosis Vaccine

Significant progress has been attained in the development of oral plant-based vaccine candidates against porcine cysticercosis (Table 3). An oral vaccine against cysticercosis was developed expressing the three peptides (KETc1, KETc12, and KETc7) that constitute the injectable vaccine against pig cysticercosis named S3Pvac. When parenterally applied, synthetically and recombinantly expressed S3Pvac reduced by 50% the number of infected pigs, and by 80–90% the number of established cysticerci under natural conditions of transmission (Huerta et al. 2001; Morales et al. 2008). To develop the oral version of the vaccine, the three peptides were expressed in three independent papaya embryogenic cell lines, obtained by bioballistics at the nuclear level under the CaMV35S promoter (Hernández et al.

Table 2 Experiences in immune response induced by oral vaccination with transgenic plants

Disease	Antigen	Plant	Dose	No. doses (interval)	Immune response	References
Gastroenteritis	LT-B	Potato tubers	20–50 μg	3 (wk)	Specific IgG and IgA Abs; partial protection	Mason et al. (1998)
Hepatitis	HBsAg	Potato tubers	20 μg	3 (wk)	Specific IgG Abs on day 36–50 after first feeding	Rukavtsova et al. (2015)
Dengue	cEDIII-CTB	Rice calli	100 μg	4 (0, 2 4, 9 wk)	cEDIII specific IgG and IgA Abs; splenic T cell proliferation	Kim et al. (2016)
Fasciolosis	CPFhW-HBcAg	Lettuce leaves	10 μg	2 (0, 4 wk)	65.4% protection; specific IgG1 and IgM Abs; increased blood CD4 + and CD8+	Kesik-Brodacka et al. (2017)
Cysticercosis	KETc1, KETc7, KETc12	Papaya calli	0.1–10 μg	2 (1, 10 days)	55–89% protection; specific IgG Abs; CD4 and CD8 proliferation	Fragoso et al. (2017)
Rheumatoid arthritis	APL-CII	Rice seeds	100–120 μg	14 (daily)	IL-10 production in spleen; reduced joint inflammation	Lizuka et al. (2014)

LT-B: *E. coli* heat-labile enterotoxin B subunit; HBsAg: surface hepatitis B antigen; cEDIII-CTB: envelope protein domain III (cEDIII) fused to cholera toxin B subunit; CPFhW-HBcAg: Cysteine proteinases from *F. hepatica;* APL-CII: Altered peptide ligands fused of type II collagen; wk: weekly; Abs: antibodies

Table 3 Transgenic plants and antigens evaluated to design an anti cysticercosis vaccine

Plant	Antigen	Expression Vector	Transformation	Specie	/Pathogen/ #immunizations/ route	% Protection	References
Carica papaya L.	KETc1, KETc12, KETc7	pUI235–5.1	Bioballistics	Mouse	*T. crassiceps*/ Two/sc		Hernández et al. (2007), Rosales-Mendoza et al. (2012)
				Rabbit	*T. pisiformis* Two/oral		Betancourt et al. (2012)
				Mouse	*T. crassiceps*/ Two/oral	55–66	Fragoso et al. (2017)
				Pig	*T. solium*/Two/ oral	ND	
Nicotiana tabacum	KETc1 KETc12 KETc7, GK1 HP6/TSOL18	pBI-Helios2A polyprotein system	*A. tumefaciens GV3101* strain	Mouse	Three/sc	ND	Monreal-Escalante et al. (2015)
Daucus carota L.	HP6/TSOL18	pBin	*A. tumefaciens* GV3101 strain	Mouse	*T. crassiceps*/ Two/oral	80%	Monreal-Escalante et al. (2016)

sc subcutaneous

2007). The combination of three embryogenic transgenic papaya callus lines was designated as S3Pvac-papaya. The expression of the respective peptide in each clone was confirmed at the transcriptional level by RT-PCR. Soluble extracts from the transgenic papaya clones were found to be immunogenic when subcutaneously administered to mice. Indeed, all three clones expressing the vaccine peptides induced high levels of protection against murine cysticercosis when injected to mice (Hernández et al. 2007).

Furthermore, orally administered S3Pvac-papaya was found to be protective against murine and rabbit cysticercosis caused by *T. crassiceps* and *T. pisiformis,* respectively (Betancourt et al. 2012; Fragoso et al. 2017). The protective properties of the vaccine were maintained when formulated with different excipients that could eventually be attractive for pigs (Fragoso et al. 2017).

The effectiveness of the vaccine against these highly predictive experimental models let us consider its usefulness to be employed for pig cysticercosis prevention. To further examine this possibility, the immunity of pigs orally vaccinated with S3Pvac-papaya was explored. Oral vaccination with S3Pvac-papaya elicited an exacerbated humoral and cellular response in pigs (Fragoso et al. 2017).

Given the promising potential of papaya-made *T. solium* antigens, their expression in plant systems has been expanded to add new advantages to the plant-made vaccine candidates. The simultaneous expression of *T. solium* antigens in a single plant line would facilitate vaccine formulation; thus, innovative approaches have been recently explored to address this objective. An alternative Helios2A polyprotein system was developed, which relies on the use of the 2A sequence (LLNFDLLKLAGDVESNPG-P) of the foot and mouth disease virus that is placed between each of the antigens in a translational fusion arrangement. During the translation process of the polyprotein-encoding mRNA coding for the target antigens, the 2A sequence induces self-cleavage events by modifying the activity of the ribosome to allow hydrolysis of the ester linkage 2A-tRNAgly to be released, while the translation of the downstream product continues (Ryan and Drew 1994). Thus, this approach would allow the production of a multicomponent vaccine through the insertion of a single expression cassette coding for the polyprotein arrangement (Liu et al. 2007; Minskaia et al. 2013; Minskaia and Ryan 2013). Following a 2A-based polyprotein expression approach, a new multicomponent vaccine called Helios-2A, comprising the KETc1, KETc12, and KETc7 peptides from the S3Pvac along with the TSOL18/HP6-TSOL protective antigen was generated. The latter was included to assess whether vaccine efficacy is improved, since it has been reported as a highly protective antigen against porcine cysticercosis (Lightowlers et al. 2016), The Helios-2A system allowed the successful expression of the KETc1, KETc12, KETc7, GK1 (a short protective sequence inserted in the KETc7 peptide), and Tsol18/HP6 individual antigens in tobacco plants transformed with *Agrobacterium tumefaciens* at the nuclear level using the CaMV35S promoter. Interestingly, plant-made Helios-2A antigens were recognized by cerebral spinal fluid of neurocysticercosis patients and induced humoral responses in mice upon subcutaneous immunization (Monreal-Escalante et al. 2015). Although the efficacy of the Helios-2A is still under assessment, it is proposed as a highly convenient

vaccine that could be produced by propagating and characterizing a single transformed line (instead of the three lines required to formulate S3Pvac-papaya), and possibly conferring higher protection than the original S3Pvac vaccine.

Another innovation developed by our group consisted in the use of carrot cells as expression host to produce anti-cysticercosis vaccines. Carrot cell lines constitute a pioneering case in the molecular pharming arena, since the first commercialized plant-made biopharmaceutical was produced in this system. Thus, carrot was adopted to produce a candidate vaccine against cysticercosis based in cell lines expressing the TSOL18/HP6-Tsol antigen. Carrot lines were obtained by *A. tumefaciens* transformation at the nuclear level to express the TSOL18/HP6-Tsol antigen under the control of the CaMV35S promoter. Upon oral immunization with carrot-made TSOL18/HP6-Tsol, mice developed humoral responses and were protected against *T. crassiceps* challenge (Monreal-Escalante et al. 2016). Immunization trials to compare the efficacy of this vaccine with that of S3Pvac are ongoing.

Looking to enhance antigen yields, transplastomic approaches have been implemented to produce the target *T. solium* antigens. The S3Pvac-papaya components were produced along with the TSOL18/HP6-Tsol antigen in tobacco plastids.

Synthetic operons under the control of the Prnn promoter led to the expression of individual target antigens through a single transformation event. Chloroplast-made antigens retained their immunogenic properties, as revealed by immunization experiments in mice. The immunoprotective properties of this transplastomic vaccine are currently being assessed (Rosales-Mendoza et al., unpublished). As an additional advantage, this vaccine offers enhanced biosafety with respect to the nuclear transformed plants, since plastomes are maternally inherited, and thus transgene transmission via pollen is avoided. Thus, the transplastomic approach is likely to yield an optimized anti-cysticercosis vaccine; however, its detailed characterization and the assessment of its protective efficacy are still in progress.

2 Conclusions and Perspectives

Cysticercosis control is theoretically possible, and the disease was declared to be eradicable by the International Task Force for Disease Eradication in 1993. However, *T. solium* cysticercosis persists to date, and new cases are continually reported in non-developed countries, where the parasite life cycle is well established, and also in developed regions due to immigration of infected individuals. Control strategies based on mass-treatment for human taeniasis in identified transmission foci have been proposed by WHO (2010) and the Pan American Health Organization. The inclusion of an effective oral, low-cost vaccine that could be administered directly by pig owners may significantly improve the effectiveness of a control program. The production of anti-cysticercosis vaccines using plants can accomplish this goal. Substantial advances have been achieved over the last

10 years in this area. Both nuclear and transplastomic approaches have been assessed to test the biosynthetic capacity of plants to produce immunoprotective *T. solium* antigens. S3Pvac-papaya vaccine was a pioneering case for a vaccine tested in the field (Hernández et al. 2007); this first experience demonstrated that plants are promising biofactories for anti-cysticercosis vaccines, and justify the projection to generate other vaccine candidates, facilitate vaccine formulation, and maximize antigen productivity. The promising results reported in pigs prompt us to start the scale-up process to produce an oral vaccine in airlift bioreactors and obtain enough material for conducting field trials. On the other hand, a vaccine based in carrot cell lines expressing the TSOL18/HP6-Tsol provided the first evidence on the production of the functional antigen at appropriate levels to immunize mice. Since TSOL18/HP6-Tsol also confers immunoprotection against *T. saginata* and the S3Pvac peptides are highly conserved in this parasite, this vaccine candidate will allow us not only to perform studies on its role as a supplementary antigen for the S3Pvac vaccine, but also to develop a new anti-*T. saginata* vaccine for cows and cattle (Parkhouse et al. 2008).

Vaccines produced in tobacco, either by nuclear or plastid expression, exemplify the potential of synthetic polycistrons and viral sequences to engineer plant cells as efficient biofactories to produce multicomponent vaccines in a single transformed line. This expression modality will greatly facilitate vaccine formulation, since the upstream process will deal with a single seed stock, and during downstream processing a single line will be used for antigen quantification and encapsulation prior to dosage. Preclinical evaluation of these 'single line' vaccine candidates will be completed soon, and will provide the basis for field evaluations in pigs.

In conclusion, plants have proven to be suitable platforms to produce anti-cysticercosis vaccines, and promising prospects are being projected in terms of field evaluations and the development of innovative candidates, based on alternative expression approaches. Such plant-based vaccines will be valuable tools to control cyticercosis especially in poor countries, since formulations based on freeze-dried plant biomass have very low production costs and lower logistic costs, since they are easy to apply and do not require purification, cold-chain, sterile devices nor trained personnel to be applied. Altogether, these features would make a more robust and easier to handle vaccine (Hirlekar and Bhairy 2017).

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