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Review

Improvement of the synthetic tri-peptide vaccine (S3Pvac) against porcine *Taenia solium* cysticercosis in search of a more effective, inexpensive and manageable vaccine

Edda Sciutto [a,*], Gabriela Rosas [b], Marisela Hernández [a], Julio Morales [c], Carmen Cruz-Revilla [a], Andrea Toledo [a], Karen Manoutcharian [a], Goar Gevorkian [a], Abel Blancas [a], Gonzalo Acero [a], Beatriz Hernández [d], Jacquelynne Cervantes [a], Raul J. Bobes [a], Fernando A. Goldbaum [e], Mirna Huerta [f], Alicia Diaz-Orea [g], Agnes Fleury [h], Aline S. de Aluja [c], Jose Luis Cabrera-Ponce [i], Luis Herrera-Estrella [i], Gladis Fragoso [a], Carlos Larralde [a]

[a] *Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, México, D.F. 04510, Mexico*
[b] *Facultad de Medicina, Universidad Autónoma del Estado de Morelos, 62210 Cuernavaca, Morelos, Mexico*
[c] *Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, México, D.F. 04510, Mexico*
[d] *Faculta de Medicina, Universidad Nacional Autónoma de México, México, D.F. 04510, Mexico*
[e] *Fundación Instituto Leloir, Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, Av. Patricias Argentinas 435, Buenos Aires 1405, Argentina*
[f] *Facultad de Medicina, Universidad Autónoma del Estado de Puebla, Puebla, Mexico*
[g] *Centro de Investigación Biomédica de Oriente, Instituto Mexicano del Seguro Social, Puebla, Mexico*
[h] *Instituto Nacional de Neurología y Neurocirugía, SSA, Insurgentes Sur 3877, México, D.F. 14269, Mexico*
[i] *Unidad de Biotecnología e Ingeniería Genética de Plantas, Unidad Irapuato, Libramiento Norte, km 9.6, Irapuato, Guanajuato, Mexico*

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Abstract

Vaccination of pigs may curtail *Taenia solium* transmission by reducing the number of cysticerci, the precursors of adult intestinal tapeworms in humans. Several antigen preparations induce protection against porcine cysticercosis in experimental settings but only one subunit vaccine (S3Pvac) has been tested and proved effective in the field against naturally acquired disease. Besides improving of the vaccine's effectiveness, significant reductions in production costs and in the logistics of its administration are necessary for the feasibility of nationwide control programs.

This review highlights the development of several versions of S3Pvac aimed to increase effectiveness, reduce costs and increase feasibility by novel delivery systems and alternative routes of administration.

Keywords: *Taenia solium*; Cysticercosis; Vaccination; S3Pvac improvement; Control

Contents

* Corresponding author. Tel.: +52 55 56223153; fax: +52 55 56223369.
E-mail address: edda@servidor.unam.mx (E. Sciutto).

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1. The dimensions of the task

In order for a vaccine against porcine cysticercosis to significantly reduce transmission of *Taenia solium* in humans and pigs living in the poorest areas of the Third World, where the parasite thrives, the vaccine must not only be immunologically effective but must also be inexpensive and simple to administrate to millions of rustically bred pigs, which are renewed each year and are dispersed in thousands of villages across large and variable geographic territories.

2. Immunological effectiveness

2.1. Effective vaccine candidates against experimental *T. solium* porcine cysticercosis

Vaccination is one of the most effective biotechnological tools for the control of bacterial and viral infections [1–3]. In contrast with vaccines against various protozoan parasite infections which have faced serious difficulties [4–6], the vaccines against infections caused by the metacestode stage of several cestodes, have consistently proved effective in experimental conditions. Indeed, several effective vaccine candidates have been developed since the pioneering work of Rickard and White [7] against the larval phase of *Taenia ovis* [8]. There are currently a number of reports about successful vaccination against metacestode disease caused by *Taenia saginata* [9,10]; *T. solium* [11–15]; *Echinococus granulosus* and *E. multilocularis* [16–18]; *Taenia crassiceps* [19–24] and *Hymenolepis nana* [25]. Two parameters are usually employed to evaluate vaccine efficacy against porcine cysticercosis: the number of infected pigs (detected by necropsy or by tongue inspection) and the number of cysticerci identified in each carcass (intensity). Tongue inspection is notoriously less sensitive than thorough histological dissection at necropsy [26,27]. In many instances, however, either because of the large number of pigs involved in the study or because of the owners' refusal to sacrifice his pigs, or because diagnosis of antibody levels in vaccinated animals is ambiguous, tongue inspection is the only plausible way to estimate the protective effects of the vaccine. The vulnerability of the metacestodes to the acquired immune response induced by vaccination may be related to a number of factors not yet extensively explored, among which the Th1, Th2 [28–32] and innate-immunity profiles seem to be involved [33]. Host and parasite-related factors (i.e., genetic background [34,35] and sex [36]) may also be involved in the effectiveness of vaccination. In addition, the high vulnerability of infective oncospheres to antibodies could also underlie the high efficacy of vaccination against taeniid cestodes [37,38].

In 1983, Molinari et al. reported the first vaccine candidate against porcine cysticercosis, based on a total extract from *T. solium* cysticerci [11]. Later on, many other vaccine candidates were developed by purification of *T. solium* and other taenids' cross-reacting cysticercal antigens in parasites recovered from naturally or experimentally infected hosts [11,12,20,39–41]. Afterwards, other successful efforts were made to identify and produce subunit vaccines in search of a stable product with high and uniform immunogenic activity (Table 1). High levels of protection were obtained using different parasite antigens from different stages of parasite development, i.e., oncospheres [13], cysticerci [11,39,40] and from homologous and or heterologous cysticerci (Table 1).

As is also shown in Table 1, high protection levels against experimental challenge were obtained using total extracts, vesicular fluid, semi-purified and recombinant or synthetic antigens. Good results were also obtained using DNA vaccination (Table 2). Worth noting is that all but one purified or recombinant vaccine candidates have been tested only under experimental conditions. Only S3Pvac has been tested twice against naturally acquired porcine cysticercosis and its efficiency has been measured by tongue inspection because of the large numbers of pigs included in the study. Some results were also obtained by necropsy but in fewer animals. Postmortem studies in the field meet with the extreme difficulties in programming the times of slaughter and necropsy inspection on account of the harsh conditions prevailing in the rural endemic areas of underdeveloped countries, where need of nourishment or local festivities frequently interfere with the

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Table 1
Listing of results in vaccination against *Taenia solium* porcine cysticercosis

Source of antigens + adjuvant	Vaccine			Diagnosis	Protection level (% reduction)	Challenge (per pig)	Reference
	Dose (per pig)	Number	Via				
T. solium excretory–secretory antigens + FCA	2.5 ml + 2.5 ml	1	n.r.	Necropsy	95% cysticerci	15,000 eggs	[42]
T. solium cysticerci extract	250 μg/pig	3	i.m.	Necropsy	74% cysticerci	Field trial	[11]
Taiwan *Taenia* frozen oncospheres + FCA	1.6×10^4	1	s.c.	Necropsy	50% infected pigs, 98% cysticerci	1.6×10^4 *T. Taiwan* eggs	[43]
Korea *Taenia* frozen oncospheres + FCA	1.6×10^4	1	s.c.	Necropsy	33% infected pigs, 99% total cysticerci	1.6×10^4 *T. Taiwan* eggs	[43]
T. saginata asiatica frozen oncospheres + FCA	1.6×10^4	1	s.c.	Necropsy	33% infected pigs, 99% cysticerci	1.6×10^4 *T. Taiwan* eggs	[43]
T. solium frozen oncospheres + FCA	1.6×10^4	1	s.c.	Necropsy	100% infected pigs, 77% cysticerci	1.6×10^4 *T. Taiwan* eggs	[43]
Taenia crassiceps extract + FCA	400 μg		i.m.	Necropsy	50% cysticerci	2.5×10^5 eggs	[12]
T. solium cysticerci extract	250 μg		i.m.	Tongue	100% infected pigs	Field trial	[39]
T. solium cysticerci extract	150 μg		i.m.	Tongue	82% infected pigs	Field trial	[40]
T. solium scolex extract + FIA	1st (3 mg); 2nd and 3rd (300 μg)	3	s.c.	Necropsy	71% cysticerci	10^4 eggs	[41]
T. solium scolex extract + *Corynebacterium parvum*	1st (3 mg); 2nd (300 μg)	3	s.c.	Necropsy	75% cysticerci	10^4 eggs	[41]
Three synthetic *T. crassiceps* peptides + S	250 μg/peptide	2	s.c.	Necropsy	98% ves cysticerci	Field trial	[14]
Three synthetic *T. crassiceps* peptides + S	250 μg/peptide	2	s.c.	Tongue	70–80% infected pigs	Field trial	[44]
TSO18-GST + Quil A	200 μg	2	i.m.	Necropsy	100% cysticerci	40×10^3 eggs	[15]
TSOL45-1A-GST + Quil A	200 μg	2	i.m.	Necropsy	0%	40×10^3 eggs	[15]
TSO18-GST + TSOL45-1A-GST + Quil A	200 μg each	2	i.m.	Necropsy	95%	40×10^3 eggs	[15]
TSO18-GST + Quil A	200 μg	3	i.m.	Necropsy	99%	9×10^3 eggs	[15]
TSOL45-1A-GST + Quil A	200 μg	4	i.m.	Necropsy	97%	9×10^3 eggs	[15]
TSO18-GST + Quil A	200 μg	2	NR	Necropsy	99.9% cysticerci	Gravid proglottids	[45]
TSOL45-1A -GST + Quil A	200 μg	2	NR	Necropsy	97% cysticerci	Gravid proglottids	[45]
T. crassiceps 56 + 66 + 74 kDa proteins + FCA	150 μg	2	i.m.	Necropsy	97% cysticerci, 86% infected pigs	2.5×10^5 eggs	[20]
Recombinant phage (KETc1 + KETc7 + KETc12 + GK1)	4×10^{11} recombinant phages	2	i.m.	Necropsy	97% ves cysticerci, 95% total cysticerci	17×10^3 eggs	[46]
Recombinant phage (KETc1 + KETc7 + KETc12 + GK1)	4×10^{12} recombinant phages	2	Oral	Necropsy	89% ves cysticerci, 42% cysticerci	17×10^3 eggs	[46]

FCA: freund complete adjuvant; FIA: freund incomplete adjuvant; S: saponin; NR: not reported; tongue: tongue inspection; ves cysticerci: vesicular cysticerci.

Table 2
Listing of results in DNA vaccination against *T. solium* porcine cysticercosis

Source of antigens + adjuvant	Vaccine			Diagnosis	Protection level (%)	Challenge (# eggs/pig)	Reference
	Dose (per pig) (μg)	Number	Via				
T. solium B + PV93	100	3	i.m.	Necropsy	85–99% cysticerci	18×10^2	[47]
cC1 + pcDNA3	500	3	i.m.	Necropsy	73% cysticerci	2×10^4	[48]
pVAX-S-deltaC-3n: hepatitis B core antigen particle + S3Pvac + IL2 signal peptide	500	2	i.m.	Necropsy	83% cysticerci	2×10^4	[49]
pcDNA3-cC1 + (GST-cC1 + FIA)	500 + 200	3	i.m.	Necropsy	Challenged (weeks after vaccination) 6 weeks, 85%; 12 weeks, 77%; 20 weeks, 72–79%	2×10^4	[50]
pcDNA3-B *T. solium*: antigen B + pcDNA3.1	1000 + 200	1	i.m.	Necropsy	92.6% cysticerci, 4/5 pigs totally protected	2×10^4	[51]

strict following of the program [52]. Nonetheless, note in Table 1 that vaccination effectively reduced the intensity of the pigs' natural infection above 90% and to a lesser extent (∼50–70%) the percent of totally protected pigs [14].

3. Identification of the S3Pvac components

3.1. Usefulness of T. crassiceps murine cysticercosis as an experimental model for the identification of promising vaccine candidates against T. solium porcine cysticercosis

Experimentation leading to a vaccine candidate against porcine cysticercosis is costly, difficult and slow if performed in pigs. On the other hand, experimental murine cysticercosis caused by *T. crassiceps* is a comparatively inexpensive and fast alternative approach to test antigens to be used as vaccine candidates against *T. solium* pig cysticercosis. The similarities in antigen composition among these two cestodes [53] and others [44,54,55] have allowed the use of antigens from one species to be applied to studies of a different one [12,13,19,24,56]. In addition, *T. crassiceps* cysticerci can rapidly reproduce asexually in the peritoneal cavity of mice, and intensity can be counted in each infected mouse, a convenient property that facilitates the assessment of vaccination effects, as well as the effects of sex, age, stress and genetic background [19,34,57]. Murine cysticercosis has demonstrated the value of investigating promising antigens for vaccination [12,19] and of testing different vaccination approaches [23,58,59].

3.2. Development of the synthetic S3Pvac vaccine

Three recombinant antigens (KETc7, KETc1 and KETc12) against cysticercosis were identified in a cDNA library of *T. crassiceps* metacestodes using specific antibodies against two antigen fractions that induced high levels of protection against murine and pig cysticercosis [20]. These recombinant antigens were also recognized by sera from *T. solium*-infected pigs and were shown to induce protective immunity against murine cysticercosis [20].

The recombinant antigen KETc7 codes for a polypeptide of 100 amino acids. Its protective capacity has been confirmed using DNA vaccination [23,60,61]. Furthermore, three putative epitopes were identified in the KETc7 sequence by computer-aided prediction of antigenicity and were synthesized in solid-phase [62]. The protective capacity of the three synthetic peptides produced was tested in murine cysticercosis. One of these peptides of 18 aa, produced in the linear form (GK1), induced the highest levels (96–99%) of protection against murine cysticercosis [21]. In addition, based on the complete amino acid sequence encoded by selected clones, two peptides designated as KETc1 (12 aa) and KETc12 (8 aa) were synthesized. Both peptides also induced high levels (66.7–100% and 52.7–88.1%, respectively) of protection against murine cysticercosis [22]. It should be noted that these three peptides belong to native antigens present along the different stages of *T. solium* parasite development (egg, tapeworm and cysticercus) [21,22], and that are exposed in different anatomical structures, thus representing different immunological targets in the parasite (Figs. 1 and 2), a fact that widens the spectrum of action of the vaccine, offering the possibility of being used to prevent the intestinal tapeworm stage of the parasite [63].

3.3. Field trials of S3Pvac against naturally acquired porcine cysticercosis

The field trial is an indispensable requirement before the extensive application of a vaccine to prevent naturally acquired disease in the authentic subjects that are continuously exposed to high risk of infection. The stress on realistic testing is of particular relevance in pig cysticercosis because of the many variables involved in the dynamics of transmission. It does not suffice that the antigen(s) are effective in highly controlled experimental conditions. Experiments usually employ a low number of pigs, all of uniform genetic background, of similar age and gender, in superb health and relaxed conditions and very well nourished with balanced

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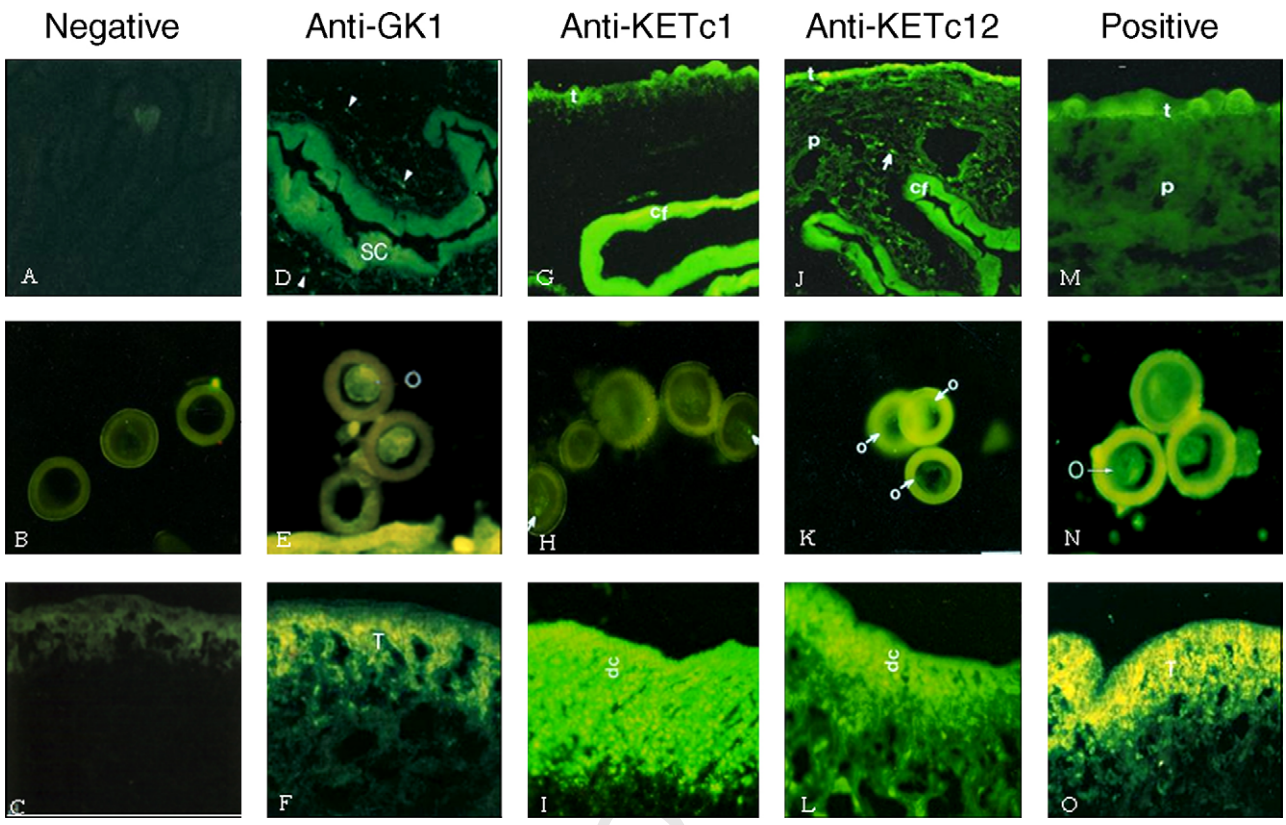

Fig. 1. Immunofluorescent staining of *Taenia crassiceps* (A, C, E, and G) and *Taenia solium* (B, D, F, and H) cysticerci. Sections of 6 mm were processed and incubated with pooled sera from non-infected mice (A and B), *T. crassiceps*-infected mice (C and D), KETc1-immunized (E and F) and KETc12-immunized (G and H) and GK1-immunized (I and J) mice. The tegument (t) and the parenchyma (p) are evident in both cysticerci (C and D). In *T. crassiceps* cysticerci (E), KETc1 antigen shows a protruding and intensely positive signal in the tegument, while in *T. solium* cysticerci (F) it is clearly evident in the cuticular folds of the spiral canal (cf) and also in the tegument (t). KETc12 is quite abundant in both metacestodes; it is evident in the tegument and in the parenchyma of *T. crassiceps* (G) as well as in the tegument, parenchyma, and flame cells (arrows) of *T. solium* (H). GK-1 is intensively expressed in the tegument (T) of *T. crassiceps* cysticerci (I) and strongly expressed in the cuticular folds of the spiral canal (SC) and flame cells (arrows). Bar, 40 mm.

foods and proper feeding schedules. Furthermore, experimental protocols on pigs generally use a single infection challenge with limited numbers of eggs, all produced by a single tapeworm, and the infection is studied at a single time after infection. The real conditions in the field differ in all the above mentioned variables, with significant impact on the probability of infection, on its intensity and on the likelihood of the host developing a competent immune reaction. In the rural areas of Mexico, and perhaps in other endemic countries, rustic pigs are genetically vastly heterogeneous besides being malnourished, stressed and exposed to various other diseases. During their usual 1 year long perilous existence, rural pigs are exposed to *T. solium* egg ingestion on multiple occasions and in diverse amounts, probably produced by different tapeworm specimens. Thus, a field trial implies a number of circumstances which are impossible to reproduce experimentally. These complex and interactive circumstances can affect the host's immunity that underlies vaccine effectiveness. Surely, field trials of a vaccine against porcine cysticercosis are extremely difficult, dangerous, time consuming, logistically complicated and costly. They require of a team of workers endowed with various abilities, including the hunting, seizing, restraining and injecting of fiercely defensive animals, and develop specialized social skills to communicate with local inhabitants of a different culture. Not the least of the field trials' difficulties is to rescue enough valid data from a process which, once on its way, meets with so many varied and uncontrollable events. Especially those related with the disappearance of many of the included pigs, victims of their predators, of other diseases and of the owners' sudden needs of food or money. Nonetheless, field trials are the closest approximation to a veritable assessment of a vaccine's potential effect in preventing naturally acquired porcine cysticercosis in endemic areas.

S3Pvac was evaluated in the field against naturally acquired *T. solium* porcine cysticercosis. Two different trials were performed in two rural communities in Mexico.

The first trial was carried out in Tepetzetzintla, Puebla [14,64]. Pigs of mixed genetic breeds were reared in the communities. Pigs were immunized twice with S3Pvac using saponin as an adjuvant whilst controls received only saponin. A total of 278 piglets were distributed in pairs (one immunized pig/one control pig) among households of the community with the understanding that the pigs would be reared

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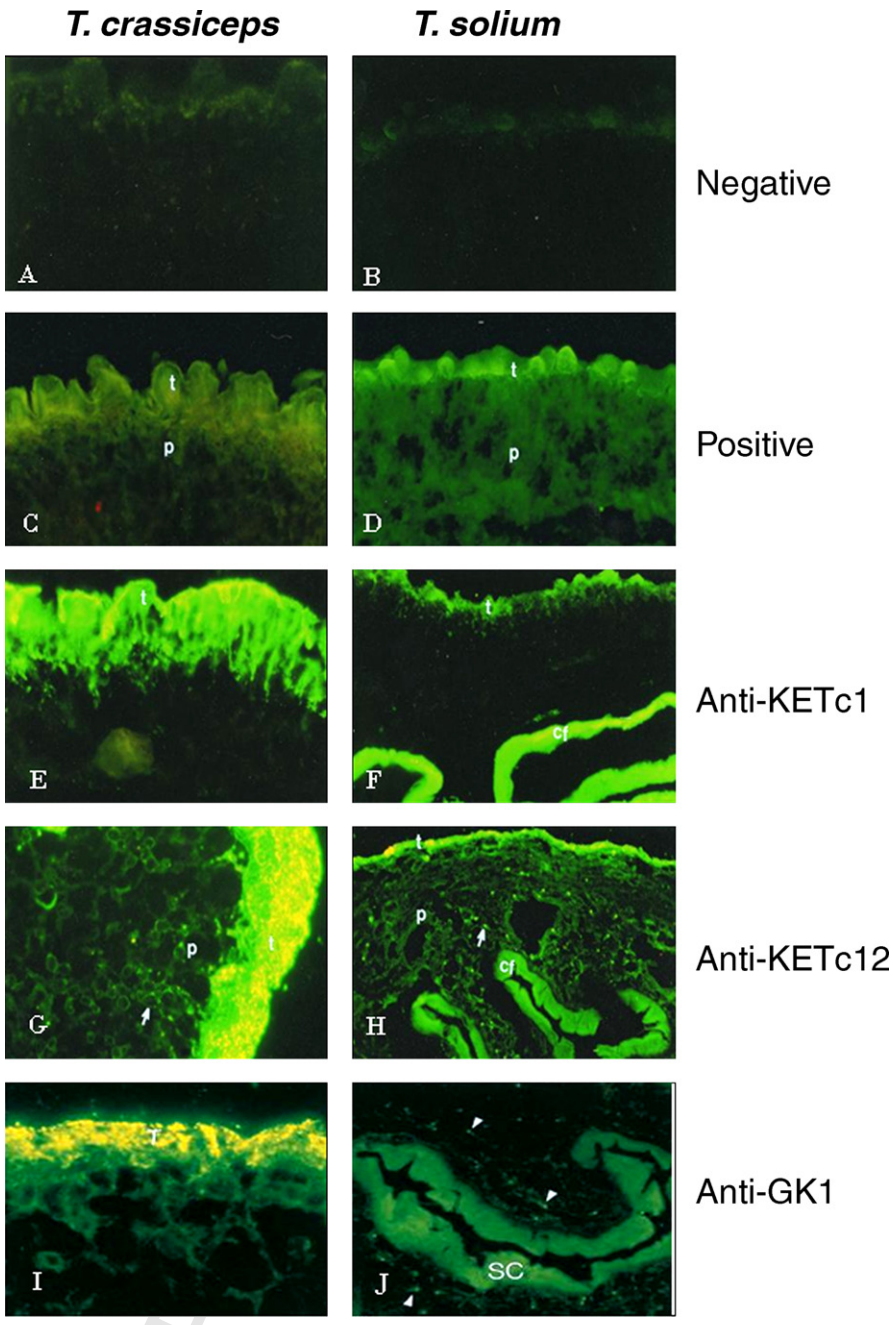

Fig. 2. Immunofluorescence staining of the *T. solium* cysticerci (A, D, G, J, and M), oncosphere (B, E, H, K, and N) and proglottid tegument (C, F, I, L, and O). Sections of 6 mm were processed and incubated with pooled sera from non-infected mice (A, B, and C), *T. crassiceps*-infected mice (M, N, and O), GK1-immunized (D, E, and F) and KETc1-immunized (G, H, and I) and KETc12-immunized (J, K, and L) mice. In cysticerci, all peptides are present in the spiral canal (CF and sc). KETc1 and KETc12 are in tegument (t) but only KETc12 is in parenchyma (p). GK1 is the most abundant peptide expressed in the oncospheres (o), and KETc1 and KETc12 are lightly presented. Also, the three peptides are present in the distal cytoplasm region (dc) of the tegument of adult tissue. Bar, 40 mm.

following traditional community methods. Thirty-eight pigs were lost before sacrifice because of different uncontrollable causes (malnutrition, infectious diseases, snake bites, scorpion stings, necessary sale, prey of predators, village festivities). The remaining 240 pigs were sacrificed at 10–12 months of age and thoroughly dissected in search of cysticerci. The number of cysticerci found in each half carcass was quantified and parasites were classified as vesicular or calcified according to their macroscopic and microscopic aspect. As shown in Table 3, the vaccine reduced the number of infected pigs by 50% and lowered parasite intensity from 66,563 to 1369 (98%), especially because there were no pigs heavily infected. Reducing the number of cysticerci is extremely important since each cysticercus is potentially

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Table 3
Protective capacity of the S3Pvac in the two different field trials performed in Mexico

Communities (state)	Number of pigs	Level of protection (%)		Reference
		Pigs totally protected	Reduction in parasite load	
Huatlatlauca, Tepezezintla, Puebla	240	50[a]	97 (66565/1364)[*]	[14]
Cuentepec, Morelos	166	70[b]	100 (29/0)[**]	[44]

[*]Total number of cysticerci recovered in 120 controls and 120 vaccinated pigs, [**]and in the tongue, masseters and diaphragm of 20 controls (2 pigs infected) and 20 vaccinated pigs.
[a] Percent of pigs totally free of cysticerci by necropsy.
[b] Percent of non-cysticercotic pigs by tongue inspection.

capable of reaching the tapeworm stage. It is also interesting that S3Pvac damaged over 80% of the cysticerci established in the immunized pigs [14].

In the second trial the protective effect of S3Pvac was assessed by tongue inspection in 166 pigs. Only 40 pigs included in the study underwent inspection by necropsy (Table 3). This trial was a 5-month S3Pvac vaccination program which included 80% of new-born piglets in the rural community of Cuentepec, Morelos. Cysticercosis was found in the tongues of 4% and 3% of the one- and two-dose groups, respectively, both significantly lower than the 14% found in sentinel pigs before the trial, and not quite significantly different from the 10% (2/20) found in the very few surviving saponin controls [44]. These results support the protective effect of the S3Pvac vaccine observed in the first trial [14], overriding the differences between the villages of two different geographic areas.

Further support for S3Pvac was provided by Wu et al. [49] who inserted the KETc1 and KETc12 epitopes into the immunodominant loop of the truncated HBc149, and GK-1 epitope in its C-terminus. As shown in Table 2 a high level of protection (83%) against experimental pig cysticercosis was obtained by DNA immunization using this fused protein deltaC-3n expressed in pVAX3.0 with the signal peptide of IL-2 [49]. Considering that this study was performed using *T. solium* eggs from a different continent, the high protective response induced by vaccination indicates that S3Pvac could be helpful in spite of possible significant genetic differences between cysticerci from different continents [65,66].

As it is shown in Table 4, S3Pvac has also exhibited therapeutic properties [67], as reported for total antigens [39]. Clear evidence of the cysticidal effect of S3Pvac but not of GK1 alone has been recently published [67]. S3Pvac injection in 30-day experimentally infected pigs reduced the number of vesicular cysticerci in muscles and increased the number of damaged and necrotic cysticerci [67]. The therapeutic properties of S3Pvac add to its interest as a powerful tool that significantly interferes with the development of cysticercosis in its porcine host.

4. Reducing costs of production of the S3Pvac anti-cysticercosis vaccine

Once the protective capacity of an immunogen has been evaluated in natural conditions of transmission, the costs of its production should be considered, especially when aiming to apply it amongst the poorest sectors of developing countries [68].

To reduce the cost of the S3Pvac, a new inexpensive recombinant version of S3Pvac expressed in filamentous phages was developed (PhageCistiVac) [46]. Filamentous phages are a suitable delivery system for inexpensive massive production of the vaccine, which hence do not require additional adjuvant for immunization. The DNA that codes for GK1, KETc12 and KETc7 was inserted into a phagemid vector to express the peptides as N-terminal fusions in M13 bacteriophage major coat protein (CPVlll), which is expressed in high copy number on the phage's surface. KETc1 was displayed on phage minor coat protein pIII. The pool of the four recombinant heat-inactivated phages induced high levels of protection against experimental murine and pig cysticercosis [46]. Important progress has been made in field evaluation of PhageCistiVac in natural transmission conditions of pig cysticercosis: preliminary results indicate that it is as efficient as S3Pvac [69]. Altogether, these results endorse PhageCistiVac

Table 4
Therapeutic effect of the S3Pvac against experimental *T. solium* cysticercosis

Status of the infected pigs	Calcified cysticerci (%)	Evagination[a] (%)	Tapeworms transformation[b] (%)
Saponine	6.41 (59/1039)	70	58
S3Pvac + saponine	61.1 (344/563)	38	19

Five piglets per group were orally infected with 20,000 of *T. solium* eggs and 1 month later treated with saponine (control) or S3Pvac, three times each at a 30-day interval. Four months after the last immunization, animals were euthanized and the total number of cysticerci found in half of the carcass, plus heart, diaphragm and brain and their macroscopical aspect were recorded.
[a] In vitro evagination capacity from a total of 269 (controls) and 50 (S3Pvac) cysticerci sampled.
[b] In vivo transformation of cysticerci to tapeworms in immunodepressed hamsters orally infected with 5 cysticerci each in a total of 20 (control pigs) and 10 (S3Pvac) hamsters [67].

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as a cost-effective vaccine to be used in developing countries where cysticercosis is endemic (unit cost of production of a single dose of vaccine is US$ 0.25). This recombinant vaccine will be produced by the Biomedical Research Institute (Instituto de Investigaciones Biomédicas) of the National Autonomous University of Mexico (Universidad Nacional Autónoma de México) and will be made available at no cost for initial applications in regional control programs in Mexico.

5. Improving the feasibility of S3Pvac wide and sustained application

Scientific evidence points to vaccination as a potentially useful tool for cysticercosis prevention, and S3Pvac-based PhageCistiVac seems ready for regional application in the field. However, both vaccines – S3Pvac and PhageCistiVac – are administered by injection. This is a paramount limitation for their application on a nationwide and sustained control program. The costs and likelihood of once per year gathering and activating a number of task-forces to vaccinate the 6 millions of rustic pigs distributed throughout Mexico in thousands of small and recondite villages is inconceivable. It would be more feasible if owners could vaccinate their own pigs. The easiest way to implement such regime would be to develop an inexpensive orally administered vaccine, which could mix with the pigs' food.

5.1. Expressing the peptides on Brucella spp. lumazine synthase: a novel adjuvant and antigen delivery system that effectively induces oral immunity

The possibility of oral administration of the anti-cysticercosis vaccine was explored using a potentially appropriate delivery system of the S3Pvac peptides, as is the case of the polymeric protein *Brucella lumazine synthase* (BLS) [70]. BLS is an immunodominant *Brucella* antigen, able to generate strong humoral as well as cellular immunity against *Brucella abortus* in mice [71]. In this highly immunogenic protein that folds as a stable dimer of pentamers [72], foreign peptides and proteins may be inserted at the 10 N-terminus of BLS without disrupting its general folding [72]. The enzyme lumazine synthase from *Brucella* spp. (BLS) was evaluated as protein carrier to improve antigen delivery of KETc1. KETc1 recombinantly bound to BLS (BLS-KETc1) preserved its immunogenicity and protective capacity when injected subcutaneously with no need of adjuvant [73]. Moreover, the orally administered chimera BLS-KETc1 induced up to 98% of protection against murine cysticercosis [74]. These are promising results that increase the possibility of designing a multivalent vaccine in only one chimerical protein that displays S3Pvac epitopes simultaneously on BLS. Experiments are underway to explore this possibility as well as the effectiveness of the BLS-KETc1 oral vaccine against *T. solium* under experimental and field conditions.

5.2. Expressing the peptides on transgenic papaya embriogenic callus: a new antigen delivery system

The anti-cysticercosis peptides (KETc7, KETc1.6His and KET12.6His) were successfully expressed in transgenic embriogenic papaya clones, a novel biotechnological approach that offers a new alternative for inexpensive production and oral delivery. The vaccine peptides were expressed from transgenes stably incorporated into a host plant's nuclear genome, via particle bombardment. Several immunogenic and protective clones were identified using the murine experimental model of cysticercosis. Complete protection was induced by subcutaneous immunization with some of the embriogenic papaya clones in up to 90% of the immunized mice, higher than that expected using the respective synthetic peptides. These results point to the potential usefulness of this new version of the anti-cysticercosis vaccine. The expressed antigen can be administered without the need of additional purification and is appropriate for capsule formulation, which could be easily mixed with the pigs' food pellets for oral delivery [44].

6. The mechanisms of S3Pvac protection

It is largely assumed that the mechanisms of protection elicited by pig vaccination against *T. solium* are those of acquired immunity and of the inflammatory process. The few existing results in porcine cysticercosis show that both major mechanisms are probably involved in the protective response provoked by vaccination. S3Pvac vaccinated pigs do indeed make antibodies that react with the peptides of the vaccine and with the parasite's protein antigens [30]. Likewise, the vaccinated pigs' peripheral mononuclear cells show specific increased cellular proliferation and elevated production of inflammatory cytokines (IL-2 and IFNγ) [30]. There are also signs that the vaccine peptides exhibit adjuvant properties probably involving inflammatory mediation [75]. When the vaccine peptides are expressed in filamentous phages (PhageCistiVac), the immunized pigs respond with a mixed Th1/Th2 immune response and their PBMCs exhibit a peptide/antigen-specific proliferative response in vitro, along with IFNγ and IL-4 production [46].

The precise molecular or cellular mechanisms by which the immune response injures the parasite are not clear yet. There is a general and longstanding consensus that the early stages of metacestode and onchosphere development of various taeniid species are the most vulnerable to polyclonal antibody attack (presumably with the aid of complement), whilst fully developed metacestodes are either not damaged by these antibodies [76] or evade their harmful effects by a number of mechanisms [77]. More recent research in experimental pig cysticercosis has shown the liability of early cysticerci that develop after challenge of pigs with eggs [ ] of non-vaccinated pigs. Most developing cysticerci are found dead after 12 months of infection [78] and their destruction

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rate holds a positive correlation with antibody levels and with the appearance of eosinophils surrounding the parasite, followed by other inflammatory cells [79]. It thus seems that killing of the parasite involves immune and inflammatory processes. Moreover, it has also been found that antibodies do not have to necessarily kill the cysticerci to play a significant role in cysticercus biology: preincubation of *T. solium* cysticerci with mouse or pig anti-GK1 antibodies cripples their capacity to transform into intestinal tapeworms when placed in the intestines of hamsters [29,30].

Along their development in the intermediary host cysticerci may express epitopes with which specific antibodies may react and interfere with their proper physiological function, thus limiting their transmission dynamics by blocking their way into adult egg-producing tapeworms [29]. This antibody-mediated restriction of the tapeworm stage development may add to the understanding of why there are so many cysticerci and such few tapeworms in endemic areas [80].

7. Concluding remarks

The anti-cysticercosis vaccines based on the peptides KETc7, GK1, KETc1 and KETc12 are ready for wide and sustained application because they have met with the following requirements: (a) the peptides are present in all developmental stages of *T. solium*; (b) all versions induce effective protection in experimental conditions; (c) S3Pvac and PhageCistiVac protect rustic pigs against natural infection in highly endemic areas; (d) PhageCistiVac's cost of production is low (US$ 0. 25 per dose/pig); (e) the cost and logistic difficulties involved in nationwide vaccine administration may be significantly lowered by oral administration of BLS-KETc1, which has been shown to induce protection in mice; (f) the successful expression of the vaccine's peptides by transgenic embriogenic papaya clones and their efficacy in inducing protection in mice connects these vaccines with the high expectations of novel biotechnological solutions to vaccine production and delivery.

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