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Review article

Vaccines for prevention of cysticercosis

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Abstract

Neurocysticercosis due to *Taenia solium* infection is an important cause of human morbidity and mortality. Despite the availability of effective anthelmintics, the disease remains prevalent in many parts of the world and there is a need for new and improved measures for control of the infection. An effective vaccine to prevent infection in pigs, the parasite's natural intermediate host, would be a valuable new option to assist with *T. solium* control. Several approaches are being used currently towards the development of a *T. solium* vaccine and these approaches are reviewed briefly, with emphasis on the use of recombinant oncosphere antigens. Highly effective vaccines have been developed against cysticercosis in sheep and cattle caused by *Taenia ovis* and *Taenia saginata*, respectively. This success has encouraged the adoption of a similar strategy for *T. solium*. The recent finding that one oncosphere antigen, TSOL18, can induce complete protection against *T. solium* infection in pigs, highlights the potential for development of a practical vaccine. A vision is proposed for the development of a safe, effective, inexpensive vaccine for pigs, which can be administered in an edible form. Through an international collaborative effort, research is progressing towards the realisation of such a vaccine and its use to reduce the global burden of neurocysticercosis.

Keywords: *Taenia solium*; Cysticercosis; Vaccine; Vaccination; Oncosphere antigen

1. Introduction

Neurocysticercosis due to *Taenia solium* is well recognised as an important cause of human morbidity and mortality. Currently available measures for control of the disease are inadequate and there is a need for new and improved interventions. A strong argument can be made for vaccination playing a key role in *T. solium* control (Lightowlers, 1999). The vision to which we aspire is of a vaccine which can prevent *T. solium* being transmitted by pigs, the parasite's natural intermediate host; a highly effective vaccine, able to prevent infections in neonates as well as older pigs, an inexpensive vaccine which can be delivered via an edible biscuit, without the need for equipment or trained personnel. Through the combined use of anthelmintic treatment of tapeworm carriers, vaccination of pigs and public education about the disease, the parasite could be eradicated. This is a vision for the future, because the vaccine required does not currently exist. However, these are good reasons to believe that such a vaccine will be achievable and the recent demonstration of com-

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plete protection against cysticercosis in pigs using a recombinant oncosphere antigen (discussed below) highlights the rapid progress being made towards the development of a practical vaccine.

Several approaches are being used by different research groups towards the development of a vaccine to prevent porcine cysticercosis. These approaches are reviewed here with emphasis on the application of recombinant oncosphere antigens for vaccine development.

2. General aspects of the immunobiology of taeniid cestode infections

T. solium belongs to the cestode family Taeniidae which comprises two genera, *Taenia* and *Echinococcus*. There are a number of species, including several which infect humans, causing disease, or infect domestic livestock and cause economic loss. The economic and medical significance of these parasites has stimulated a substantial amount of research into the biology of the group, and their immunobiology in particular. Many of the immunological characteristics of the host–parasite relationship between these parasites and their natural intermediate hosts are common to many if not all of the species. The extensive early research in the field has been reviewed by Rickard and Williams (1982) and the reader is referred to this source for detailed referencing. Much of the early research was undertaken with *Taenia* species which infect laboratory animals and subsequently with *Taenia* species and *Echinococcus granulosus* in sheep (Gemmell et al., 2002). A critical finding which emerged from these studies was that immunity to re-infection plays an important role in the natural regulation of transmission of this group of parasites (Rickard and Williams, 1982). This attribute sets the taeniids apart from many other helminthic infections and provides an important advantage to those interested in developing vaccines. Host-protective immune responses are directed towards the oncosphere in the early developing embryo. Rarely has there been significant success with demonstration of effective immune responses against the mature metacestode encysted in the tissues.

Initial research towards the development of vaccines demonstrated that protection could only be achieved by exposing hosts to living parasites. A breakthrough was made by Rickard and Bell (1971) who demonstrated high levels of protection against *Taenia ovis* infection in lambs in which *T. ovis* oncospheres had been grown intraperitoneally in diffusion chambers. The pore size of there chambers was 0.2 μm, hence suggesting that subcellular or soluble antigens might be protective. Subsequently, protection was achieved in vaccine trials using cell-free antigen preparations collected by culture of oncospheres in vitro and with antigens prepared directly from oncospheres (Rajasekariah et al., 1980; Osborn and Heath, 1982; Osborn et al., 1982). The high level of effectiveness of vaccine trials using oncosphere antigens set the scene for development of practical vaccines; however, the problem which remained was in production of sufficient antigen for widespread vaccine use. The answer to this problem lay in the development of recombinant DNA technology.

3. Development of recombinant vaccines against cysticercosis in sheep and cattle

The recombinant antigen vaccine which was developed against cysticercosis caused by *T. ovis* in sheep (Johnson et al., 1989) was the first effective, defined antigen vaccine against a parasitic infection and has been recognised as a milestone in the history of parasitology (Cox, 1993). The *T. ovis* vaccine received provisional registration as a commercial vaccine in New Zealand in August 1990 although this was not followed by marketing due to commercial considerations (Rickard et al., 1995); the vaccine has, however, been used extensively as a model anti-parasite vaccine (reviewed by Lightowlers and Gauci, 2001).

Successful development of a vaccine against *T. ovis* encouraged efforts to develop similar vaccines against other *Taenia* species causing cysticercosis (Table 1). The first of these was a vaccine against *Taenia saginata* infection in cattle. The *T. ovis* vaccine arose out of extensive investigations which sought to identify native oncosphere antigens capable of inducing host-protective immunity

Table 1
Recombinant oncosphere antigens of taeniid cestodes, which have been shown to induce host-protective immune responses and an indication of the level of protection, achieved in vaccination and challenge trials in the parasites' intermediate hosts

Species	Antigen	Host specie(s)	Protection (%)[a]	Reference
T. ovis	To45W	Sheep, goat	94	Johnson et al., 1989
	To45S		87	Lightowlers et al., 1996c
	To16K		92	Harrison et al., 1996
	To18K		99	Harrison et al., 1996
T. saginata	TSA-9, TSA-18[b]	Cattle	99	Lightowlers et al., 1996b
T. solium	TSOL18	Pig	100	Flisser et al., submitted
E. granulosus	EG95	Sheep, goat, cattle	96	Lightowlers et al., 1996a
			100	Lightowlers et al., 1999
Echinococcus multilocularis	EM95	Mice	83	Gauci et al., 2002

[a] Indicates the optimum level of protection achieved in vaccination and challenge trials in the parasite's natural intermediate host species compared with challenge controls.
[b] TSA-9 and TSA-18 were found to act synergistically; results represent those of vaccination trials using the two antigens together.

(Harrison et al., 1993). These studies involved many vaccination and challenge trials in sheep which could not be envisaged for *T. saginata* infection in cattle because of the cost involved in undertaking cattle trials and because of the substantial difficulties in obtaining sufficient oncospheres for these purposes. The approach which was taken to develop a vaccine against *T. saginata* was to utilise the information which had been obtained for *T. ovis* with regard to the identity of the host-protective antigens. DNA hybridisation was able to identify the presence in the *T. saginata* genome of DNA sequences having close homology to each of the three cDNA's (45W, 16K, 18K) from *T. ovis* which each encode a host-protective antigen. These homologues were cloned from cDNA prepared using mRNA extracted from *T. saginata* oncospheres (Lightowlers et al., 1996b). Two of these *T. saginata* antigens were able to be used together to induce very high levels of protection against experimental challenge infection with *T. saginata* eggs in cattle.

4. Protection against cysticercosis in pigs using oncosphere antigens

In common with all other taeniid cestodes which have been investigated, oncosphere antigens of *T. solium* have been found to be a rich source of host-protective antigens (Pathak and Gaur, 1990; Plancarte et al., 1999; Verastegui et al., 2002). The rapid success which had been achieved with identifying host-protective recombinant antigens for *T. saginata*, encouraged the adoption of a similar approach for development of a vaccine against *T. solium* cysticercosis in pigs.

Investigations using Southern blotting with *T. solium* genomic DNA probed with cDNA's encoding host-protective antigens of *T. ovis* or *T. saginata*, identified close homologues to each of the three target genes in *T. solium* (Gauci et al., 1998; Gauci and Lightowlers, 2001) Two of the associated mRNAs from *T. solium* oncospheres have been cloned and expressed in *Escherichia coli* and the recombinant proteins used in a vaccination trial against oral challenge infection with *T. solium* in pigs (Flisser et al., submitted). In this trial, one antigen (TSOL18) achieved complete protection against the development of any cysticerci in any of the vaccinated pigs, compared with the presence of many cysticerci developing in the musculature of all control pigs. This experiment demonstrated the potential for application of recombinant oncosphere antigens for development of a practical vaccine against porcine cysticercosis.

5. Other approaches to development of a vaccine against *T. solium*

Antigens derived from the rodent parasite *Taenia crassiceps* are being used as a potential source of host-protective antigens for *T. solium*.

This concept is based on the well-established immunological cross-reactivity between host-protective antigens derived from different taeniid cestode species. Toledo et al. (1999) investigated recombinant antigens of *T. crassiceps* and identified proteins which could be used to vaccinate mice against an intraperitoneal challenge with the parasite's proliferative metacestode. Peptide epitopes were identified in the *T. crassiceps* model (Toledo et al., 2001) and these have been tested in a field trial to assess their potential for protection against *T. solium* infection in pigs (Huerta et al., 2001). Pigs were vaccinated with a combination of three synthetic peptides originating from the sequence of *T. crassiceps* antigens. Vaccinated and control pigs were allocated to village households as matching pairs in an endemic area of Mexico and necropsies performed to assess the number of cysticerci when the pigs were 10–12 months old. Huerta et al. (2001) indicate that, through this experimental design, possible variations in management and exposure levels among the different households were controlled for all pairs. The results revealed statistically significantly fewer cysticerci in the nine infected pigs in the vaccinated group compared with the 19 infected pigs in the control groups. In particular, only one pig in the vaccinated group had more than 1000 cysticerci but six pigs in the control group had more than 1000 cysts. During the experiment, 38 of the 278 experimental pigs died, however, the data provided on the remaining 240 pigs does not allow comparison of results for each pair of animals. It is unclear whether any of the results shown, particularly those from pigs in which the heavy infections were detected, were from pairs in which the second member of the pair had died and for which no data were available. Should any data from such animals have been included in the analyses, this would not be consistent with the experimental design, which was formulated to control for any variations in management and exposure levels among the different households to which the pigs were allocated. Exclusion of data from one infected pig in the control group from the analyses renders the comparison of the control and vaccinated groups as being statistically non-significant. Comparison of the number of cysticerci in the animals which were infected in the control and vaccinated groups (as distinct from the entire data-set comprising mostly uninfected animals), reveals no statistical significance between the groups. Nevertheless, the authors highlight that, based on the total number of cysticerci found in the two groups, there were 98.7% fewer cysticerci in the vaccinated group compared with the controls.

Huerta et al. (2001) used an excellent design in their field trial to assess the potential of the *T. crassiceps* antigens, however, the use of a field trial format, rather than a more controlled vaccination and experimental challenge infection trial, warrants further consideration. Clearly, a practical vaccine must be effective in the field to be useful. However, field trials may not be the most efficient method to assess the potential of antigens which have, apparently, not been tested previously in pigs since they were first described and noted as potential vaccine candidates for *T. solium* by Manoutcharian et al. (1996). Controlled, experimental infection trials allow vaccine candidate antigens to be assessed accurately and relatively inexpensively. Proven effective antigens can be optimised in controlled trials to determine attributes such as the dose of antigen, number of immunisations and duration of protection. These conditions would then form a rational basis for designing a strategy by which a vaccine candidate was tested in the field. Owing to difficulties with regulation of the level of parasite challenge which occurs in individual pigs in a field trial setting, it can be difficult to be confident about the interpretation of the trial results. It would be valuable if the proposition that high intensity infections with *T. solium* in pigs can be prevented using the antigens described by Huerta et al. (2001), were confirmed in a vaccination trial using experimental challenge infection.

Recently, Cai et al. (2001) has described the results of vaccination trials in pigs immunised with DNA constructs expressing antigen B of *T. solium*. The pigs were challenged orally with *T. solium* eggs at 14 days and 4 months after vaccination and it was found that vaccinated pigs had 84–99% fewer cysticercosis than control pigs. The possible application of DNA vaccination against *T. solium*

is particularly interesting because of the opportunities this could provide for directing the immune response both qualitatively and quantitatively by utilising a prime/boost vaccine approach or the co-delivery of cytokines as has been demonstrated for the *T. ovis* vaccine in sheep (Rothel et al., 1997, 1998; Scheerlinck et al., 2001).

6. Concluding remarks

The prospects are bright for the successful development of an effective, practical vaccine to assist with control of transmission of *T. solium*. The vision for a vaccine with the features expressed at the beginning of this article may not be attained for some time, however, the crucial beginnings have already been accomplished successfully and now vaccine delivery looms as a critical issue for implementation of a vaccine for *T. solium* cysticercosis. The vision for an edible, non-living vaccine is out of reach at the present time because none of the mucosal adjuvants which have been described show sufficient promise for practical use (Cripps et al., 2001). Nevertheless, it is likely that the substantial global interest in the field of oral vaccines will lead to effective delivery strategies which could be applied to a *T. solium* vaccine. For the time being, there is much research which needs to be undertaken to confirm the effectiveness of the vaccines which have already been described, particularly the TSOL18 vaccine which, in its initial trial, induced complete protection against infection. Many practical issues can be addressed with the TSOL18 vaccine in its present formulation, such as colostral transfer of protection, protection against infection with *T. solium* parasites from different parts of the world and demonstration of effectiveness against naturally-acquired infection. Any relevant advances in vaccine delivery strategies, which occur during this work, will be adopted and tested for effectiveness with the TSOL18 vaccine. Through collaboration with researchers from endemic countries, development of the TSOL18 vaccine will hopefully proceed rapidly. Active collaborations already exist in this regard with Ana Flisser and her colleagues in Mexico, Hector Gacia, Armando González and Robert Gilman in Peru and with André Zoli and Stanny Geerts in Cameroon. Collaborations with other researchers who wish to participate in further development of a *T. solium* vaccine, would be welcome.

A possibility which should not be overlooked is the potential for vaccination to be used in humans so as to prevent neurocysticercosis directly. With the successful development of an effective vaccine against *T. solium* in a natural animal intermediate host, it is possible that the same vaccine would be effective also in humans. The major issue here, which discourages development of a human vaccine, is the cost involved in undertaking clinical trials and in manufacture of a vaccine suitable for use in humans rather than animals. Whether vaccination for prevention of *T. solium* infection is intended for either pigs or humans, economic factors are certain to play a critical role in determining if successful scientific investigations can be translated into a practical vaccine that leads to reduced human morbidity and mortality due to neurocysticercosis.

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