Recombinant vaccine antigens are being evaluated for their ability to protect livestock animals against cysticercosis and related parasitic infections. Practical use of some of these vaccines is expected to reduce parasite transmission, leading to a reduction in the incidence of neurocysticercosis and hydatid disease in humans. We recently showed that an antigen (TSOL16), expressed in *Escherichia coli*, confers high levels of protection against *Taenia solium* cysticercosis in pigs, which provides a strategy for control of *T. solium* parasite transmission. Here, we discuss the characteristics of this antigen that may affect the utility of TSOL16 and related antigens for development as recombinant vaccines. We also report that genes encoding antigens closely related to TSOL16 from *T. solium* also occur in other related species of parasites. These highly homologous antigens have the potential to be used as vaccines and may provide protection against related species of *Taenia* that cause infection in other hosts.

The taeniid cestodes are tapeworm parasites that cause diseases known as cysticercosis and echinococcosis in animals and humans. Cysticercosis is caused by infection with the larval stage of certain species of *Taenia* following ingestion of parasite eggs that have been shed from a carrier of the adult tapeworm. Each of the several different species of *Taenia* causing cysticercosis have specific host requirements and will only infect and develop in a particular species, or closely related species, of intermediate host. An important parasite belonging to this group is *Taenia solium* which causes cysticercosis in humans and pigs. *T. solium* is a considerable cause of human neurological disease with pigs being responsible for transmission of the parasite. Vaccination of pigs to prevent transmission of *T. solium* to humans has been investigated as a measure to control the parasite in endemic areas and to reduce the incidence of human infection.

Recently, we have demonstrated the potential for a recombinant antigen, TSOL16, to confer protection in pigs against *T. solium* challenge infection. The TSOL16 antigen was expressed in *E. coli* as a glutathione S-transferase (GST) fusion protein and a maltose binding protein fusion (MBP) using pGEX and pMAL vectors, respectively. These expression systems were utilized since the affinity tags have been found to enhance expression of related parasite antigens in a soluble form, allowing affinity purification. Pigs were immunized twice with TSOL16-GST and boosted a third time with TSOL16-MBP prior to receiving a challenge infection with *T. solium* eggs. The TSOL16 antigen produced a high level of protective immunity against parasite infection, leading to a 99.8% reduction in parasites compared with control animals. The TSOL16 recombinant antigen is a protein homolog of the To16 vaccine antigen that was originally described in a related species of parasite, *Taenia ovis*. Previous demonstration of the effectiveness of To16 against *T. ovis* infection in vaccinated sheep provided initial evidence to show that a *T. solium* homolog may provide protection against *T. solium* infection. The recent findings highlight the effectiveness of
utilizing an antigen discovery strategy based on the identification of protein homologs in related parasite species.

The To16 antigen was first identified in protein extracts from the larval oncosphere of the *T. ovis* parasite by protein fractionation using SDS-PAGE. The enriched protein fractions were used to immunize sheep which were subsequently challenged with *T. ovis* eggs. The protein fraction (12–18 kDa) containing the To16 antigen protected immunized sheep against the challenge infection. Antigens from animals immunized with the protein fractions were used in immunoblot screening of a cDNA library prepared from *T. ovis* oncosphere mRNA. Following cloning of these cDNAs into bacterial plasmids and expression in *E. coli*, the recombinant antigens were assessed in vaccine trials. This approach led to identification of recombinant antigen equivalents of the protective antigens present in the parasite extracts.

The experimental approaches used to develop a vaccine against *T. ovis* have been used as a model system for the isolation of related antigens that are effective against *T. solium* infection. Compared with *T. ovis*, it is considerably more difficult to evaluate recombinant antigens in vaccine trials against *T. solium* infection. *T. solium* eggs required for pig challenge infections must be recovered from human tapeworms and individuals harboring *T. solium* are difficult to identify and, even in highly endemic regions, are quite rare in the community. For these reasons, protein fractionation of parasite extracts from *T. solium* oncospheres for use in vaccination-challenge trials in pigs have not been possible using this strategy. However, as demonstrated by cloning the TSOL16 homolog from *T. solium* and its recent assessment in a pig vaccine trial, identification of proteins that are homologous to previously proven host protective antigens is a more effective strategy. A similar strategy has allowed identification of other families of antigens in taeniid cestodes that protect livestock animals against infection.

Members of the 16K family of oncosphere proteins have been identified in *T. solium* (TSOL16), *T. ovis* (To16) and the related parasite *Taenia multiceps* (Tm16) which produces a much larger larval stage (cercaria) infecting the central nervous system of sheep and goats and, on rare occasions, humans. The Tm16 recombinant antigen, also expressed in *E. coli* as a fusion with GST, has been shown to provide vaccinated sheep with significant protection against a challenge infection against *T. multiceps*. Southern hybridization and PCR-based investigations indicate that genes homologous to the 16K family do not appear to be present in the related cestode parasites *Taenia hydatigena*, *Echinococcus granulosus* or *Taenia pisiformis* (data not shown). However, genes homologous to the 16K antigens have been identified in the related tapeworm parasites *Taenia saginata* and *Taenia asiatica* (Fig. 1). While *T. solium* and *T. saginata* encyst in pork and beef, respectively, *T. asiatica* generally encysts in the liver and visceral organs of pigs, and not in muscle tissue. The TSA16 and TASA16 genes (Fig. 1A) have a highly conserved structure containing three exons and two introns as observed in the other related genes. As with the To16, TSOL16 and Tm16 genes, exon 1 of TSA16 and TASA16 encodes a predicted secretory signal while exon 2 encodes a predicted threonine type III (FnIII) protein domain predicted fibronectin type III (FnIII) protein domain. The presence of a secretory signal within the N-terminus of this group of antigens suggests that they may be secreted by the parasite and is consistent with localization studies showing that the To16 antigen is associated with secretory blebs produced by the activated oncosphere.

Based on the previous studies which have demonstrated that the To16, Tm16 and TSOL16 antigens protect the intermediate hosts against *T. ovis*, *T. multiceps* and *T. solium* infection, the homologous antigens, TSA16 and TASA16 would be expected to protect cattle against *T. saginata* infection and pigs against *T. asiatica* infection, respectively, however these hypotheses remain to be tested.

One of the limitations in producing this group of recombinant antigens by expression in *E. coli* as fusions with GST and MBP is that the levels of production were intrinsically inadequate for large scale production. This problem has been overcome by deleting the nucleotides encoding amino acids contained within a predicted hydrophilic region at the N-terminus of the antigens. The deleted amino acids are contained within the predicted parasite secretory signal. This was also the case with TSOL16 and so the recombinant protein recently shown to protect pigs against *T. solium* infection was a truncated form of the antigen, lacking 16 N-terminal amino acids flanked by PCR amplification of a cDNA construct encoding the modified form of the antigen. This strategy is analogous to that used to optimize expression of related parasite antigens.

The successful vaccine trials using To16 and TSOL16 have demonstrated that the antigens are expressed in *E. coli* in a conformation that presents the host protective epitopes in a manner suitable for stimulating a protective immune response to the native protein on the parasite. The antigens appear to be specifically expressed in the oncosphere life cycle stage of the parasites. Localization studies of To16 and TSOL16 within the parasites have identified the antigens in the penetration glands of the oncosphere which are structures associated with infection of the host by the parasites. To16 has also been localized within secretory blebs in the activated oncosphere, a stage of the parasite’s development coinciding with host infection.

Previous studies have demonstrated that a major host protective immune mechanism stimulated by the cestode antigen appears to be antibody dependant, complement-mediated killing of the infective oncosphere. Taeniid larvae cultured in vitro, in the presence of serum from animals immunized with the recombinant antigens, are killed when complement is also present. This has been demonstrated to be a common immune mechanism in different host species against the various taeniid parasites: *T. solium* oncospheres cultured in the presence of sera from pigs immunized with TSOL18 or TSOL45 recombinant antigens. *E. granulosus* cultures exposed to sera from sheep immunized with the EG95 antigen. The identification of TSOL16 and the other protective antigen families of taeniid
cestodes provides a clear example of the effective use of the strategy whereby cloning of homologous parasite antigens leads to proven, protective vaccines. While this has been an obvious strategy for vaccine antigen discovery for some time,27 the taeniid cestode antigens provide the clearest proof of principle of the effectiveness of this strategy which can be applied to the development of anti-parasite vaccines. This strategy may provide further clues for how effective vaccines may be developed against other parasitic diseases.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
This work was funded by the National Health and Medical Research Council of Australia. Assistance with DNA sequencing by Rick Rolfe is gratefully acknowledged.
References
1. Schaar P. Taenia solium cysticercosis: an overview of global distribution and transmission. In: Singh, G, Puckhaber, A, editors. Taenia solium cysticercosis—basic to clinical science. 2002. 63-73.
2. Lightowlers MP. Evaluation of Taenia solium cysticercosis: a role for vaccination of pigs. Vet Parasitol 2010; 171, 277-287. http://dx.doi.org/10.1016/j.vetpar.2010.01.001.
3. Gauci CG, Verástegui MR, Gilman RH, Lightowlers MW. Taenia solium and Taenia evitae: stage-specific expression of the vaccine antigen genes, TSOL18, TSOL19, and homologues, in embryos. Exp Parasitol 2010; 125:172-7. PMID:20487777, http://dx.doi.org/10.1016/j.exppara.2009.10.006.
4. Gauci CG, Flisser A, Lightowlers MW, A. Taenia solium oncosphere proteins: homology in host protective Taenia evitae and Taenia saginata 38kDa antigens. Int J Parasitol 1998; 28:737-46. PMID:9506955, http://dx.doi.org/10.1016/S0020-7519(98)00016-4.
5. Gauci CG, Lightowlers MW. Alternative splicing and sequence diversity of transcripts from the oncosphere stage of Taenia solium with homology to the 40k antigen of Taenia evitae. Mol Biomed 2001; 31:123-9. PMID:11085234, http://dx.doi.org/10.1016/S0378-1119(01)00510-5.
6. Gauci CG, Verástegui MR, Gilman RH, Lightowlers MW. Immunology of parasitic infections in sheep: clinical signs and post-mortem findings. Br Vet J 1982; 138:489-94. PMID:7209943.
7. Baran GP, Warren KS. Immunology of parasitic infections of sheep. In: Verástegui MR, Lightowlers MW, editors. Strategies for control of tapeworm infections with special reference to Taenia solium. 2002: 63-73.
8. Flisser A, Gauci CG, Zoli A, Martinez-Ocaña J, Garra Rodriguez A, Dominguez-Aguilar JL, et al. Induction of protection against parasite cysticercosis by vaccination with recombinant oncosphere antigens. J Infect Dis 2004; 189:502-9. PMID:15526285, http://dx.doi.org/10.1086/423572.JID.2004.502.502.507.4.
9. Gauci CG, Flisser A, Lightowlers MW. A. Taenia solium oncospore proteins: homology and protective efficacy of TSOL18 in pigs. Int J Parasitol 2006; 36:529-33. PMID:16497417, http://dx.doi.org/10.1016/j.ijpara.2005.11.006.
10. Gauci CG, Verástegui MR, Gilman RH, Lightowlers MW. Taenia solium and Taenia evitae: stage-specific expression of the vaccine antigen genes, TSOL18, TSOL19, and homologues, in embryos. Exp Parasitol 2010; 125:172-7. PMID:20487777, http://dx.doi.org/10.1016/j.exppara.2009.10.006.
11. Gauci CG, Verástegui MR, Gilman RH, Lightowlers MW, Cisco PS, Little RJ, et al. Localization of three homologous oncosphere antigens in Taenia evitae. Int J Parasitol 2011; 41:277-89. PMID:21222242, http://dx.doi.org/10.1016/j.ijpara.2011.03.008.