Immunity and vaccine control of Echinococcus granulosus infection in animal intermediate hosts

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Abstract. Much progress has been made with characterisation of the EG95 vaccine which can be used to prevent hydatid infection in animal intermediate hosts of Echinococcus granulosus. The vaccine comprises a single recombinant oncosphere antigen and the adjuvant Quil A. It induces complement-fixing antibodies that kill the invading oncosphere early in an infection. In the majority of vaccinated animals, no hydatid cysts occur following a challenge infection. However, a small number of viable cysts may occur in some vaccinated animals. The vaccine has proved effective in vaccine trials carried out in sheep in New Zealand, Australia, Argentina, Chile and China as well as in goats and cattle. Investigations of the genetic diversity of the gene encoding EG95 have identified no unequivocal variation within the G1 strain parasites; however DNA sequence diversity within the EG95 family of genes has been found in G6/G7 parasites. GMP production scale-up of the vaccine has been undertaken in New Zealand and China and it is expected that the vaccine will be become available through these sources for implementation as part of hydatid control programs worldwide.

Since its first description in 1996, the EG95 hydatid vaccine has proven highly effective and reliable in inducing protection against challenge infection with Echinococcus granulosus in a number of species of host and in several different countries (Lightowlers et al., 1996a; Lightowlers et al., 1999; Heath et al., 2003).

Sufficient evidence has been gathered to indicate that the vaccine has great potential for practical use to reduce the level of transmission of E. granulosus and, indirectly, the burden of hydatid disease on human health. Efforts are now being directed towards the scaling-up of vaccine production such that adequate quantities of quality-controlled vaccine are available for practical use. The following discussion examines the background to development of the vaccine, investigations that have been undertaken to date and future prospects for implementation of vaccination in hydatid control programs.

Historical aspects

During the 1960’s Michael Gemmell undertook many seminal scientific investigations on the immunology of E. granulosus and other taeniid cestode infections in sheep (Gemmell, 1962, 1964, 1966, 1967; Gemmell and Soulsby, 1968). He found using Taenia ovis that oncospheres injected into the tissues of sheep were capable of causing a viable metacestode infection and that this infection could be differentiated from an infection acquired by ingestion of eggs orally (Gemmell, 1962). Sheep that had been exposed in this way to an initial infection were immune to reinfection, but only if the initial infection used living, not dead, oncospheres. These data led to the hypothesis that protection was only elicited by exposure to the living parasite. However this hypothesis was inconsistent with earlier work undertaken with taeniid cestode parasites of laboratory animals (Miller, 1931; Miller, 1932; Kan, 1934; Campbell, 1936; Campbell, 1938). Subsequently, Rickard and his colleagues showed that protection could be demonstrated in rabbits and rodents to Taenia infections following the growth of their larval stages in intraperitoneal implants having a pore size of 0.22µm (Rickard and Bell, 1971a), indicating that the protective antigens were elaborated by the living parasites but that protection did not require direct exposure to the living organisms per se. Following the successful demonstration of in vitro culture of cestode larve from the oncosphere (Heath and Smyth, 1970; Heath, 1973a,b), before long it had been shown that the supernatants from oncospheres cultured in vitro could be used to induce protection against Taenia infections (Rickard and Bell, 1971b; Heath 1973a,b; Rickard and Adolph, 1976; Rickard and Adolph, 1977). It was recognised later that oncospheres were a potent source of host-protective antigens that could be demonstrated to be present in crude, non-living extracts of the parasites and without any requirement for culture in vitro (Rajasekariah et al., 1980a, b; Osborn et al., 1981; Rajasekariah et al., 1982, 1985). Many of the host-parasite immunological characteristics of E. granulosus mirror those seen in other taeniid cestodes (Rickard and Williams, 1982; Heath, 1995) and, given these similarities, it was not surprising that oncospheres were found to be a rich source of host-protective antigens from E. granulosus (Osborn and Heath, 1982).
The demonstration of high levels of protection against Taenia infection using a non-living source of antigens paved the way for development of practical vaccines. For vaccination of large numbers of livestock, practical quantities of Taenia larva cannot be propagated in vitro and so production of sufficient amounts of vaccine antigens required the advent and application of recombinant DNA technology. In the first successful application of this technology in the field of parasitology, a recombinant vaccine against T. ovis infection in sheep was described in 1989 (Johnson et al., 1989). Close homologies of these host-protective antigens were found in Toxoplasma gondii antigens and so production of sufficient amounts of vaccine antigens required the advent and application of recombinant DNA technology. In the first successful application of this technology in the field of parasitology, a recombinant vaccine against T. ovis infection in sheep was described in 1989 (Johnson et al., 1989). Close homologies of these host-protective antigens were found in T. saginata oncospheres, and these homologous recombinant proteins were shown to be effective when used as vaccines to prevent cysticercosis in cattle due to T. saginata infection (Lightowlers et al., 1996b). Homologues of the genes encoding host-protective oncosphere antigens of T. ovis and T. saginata were not evident in the genome of E. granulosus and so the strategy that had been used successfully for T. saginata could not be adopted for development of a vaccine against hydatid disease.

Development of the EG95 vaccine against hydatid disease

While the lack of homology between the host-protective oncosphere antigens of Taenia and of E. granulosus represented a set-back to rapid development of a vaccine against hydatid disease, the known presence of highly protective antigens in E. granulosus oncospheres (Osborn and Heath, 1982) indicated that a similar strategy to that used for T. ovis for identification and cloning of protective antigens would be appropriate also for E. granulosus. Heath and Lawrence (1996) described the intensive investigations that were undertaken and which identified individual host-protective proteins from E. granulosus. Their studies also raised antibodies that were specific for particular antigens or sub-sets of antigens and these were then used to identify antigens cloned from oncosphere mRNA (Lightowlers et al., 1996a). One antigen clone, designated EG95, was found to express a protein that was capable of inducing protection against E. granulosus infection in vaccinated sheep.

EG95 vaccine proves reliable in different countries and in different animal species

Vaccine trials have now been carried in sheep against experimental challenge infection with E. granulosus in New Zealand, Australia, Argentina and Chile (Lightowlers et al., 1999; Heath et al., 2003) and on each occasion the vaccine has induced a high level of protection (Table 1). While some vaccinated animals were found to harbour hydatid cysts after the challenge infection, their number was reduced by approximately 99%, and half of the vaccinated animals were found to harbour no hydatid cysts. Heath et al. (2003) describe the levels of protection afforded by the EG95 vaccine against hydatid disease in cattle, where protection levels have been observed between 89 and 99%.

Duration of protection and field trials

Vaccine trials in sheep carried out in Argentina and China have determined that two immunisations affords protection lasting at least a year and that a third booster injection given after six - twelve months leads to solid immunity in sheep challenged 12 months or more after their first injection (Heath et al., 2003). Field trials of the EG95 vaccine have been carried out over a number of years in the Province of Xinjiang, China (Heath et al., 2003). The vaccine has proved highly effective, with annual vaccine booster injections maintaining protective immunity in field animals over a period of five years.

Vaccine scale-up

Substantial research has been undertaken in New Zealand into optimizing and scaling up GMP-quality manufacture of the EG95 vaccine. The vaccine production process has been adapted substantially from the affinity purification methodology used initially to prepare vaccine antigen (Lightowlers et al., 1996a). This technology was not easily adapted to cost-effective commercial production and new methodologies were required to meet adequate levels of vaccine production and cost. These processes are currently being applied in a purpose-built GMP vaccine production unit in Beijing. Production validation runs are in progress in the plant and it is anticipated that this will provide vaccine which will allow practical application of the vaccine in hydatid control campaigns wherever this is desired.

Studies on genetic variability in EG95

The gene encoding EG 95 has been found to be a member of a small multi-gene family (Chow et al., 2001). This fact has served to complicate the study of genetic variability in the gene encoding EG95 in different parasite isolates and E. granulosus strains. Complications arise because of the need to clearly differentiate gene sequences between those that represent EG95 per se from those of other members of the closely related gene family. There are seven genes in the genome of the G1 strain parasite isolate from which the EG95 mRNA was isolated, six of which are expressed (Figure 1; Chow et al., 2001). All members of the gene family were isolated and sequenced, providing information that has allowed the development of gene-specific primers which now enable gene-specific investigations to be undertaken (Chow et al., 2004). Zhang et al. (2003) have published some information concerning genetic variability in the EG95 gene sequences from Chinese isolates of E. granulosus. Unfortunately, the PCR-generated gene sequences determined by Zhang et al. (2003) are impossible to interpret because the RT-PCR primers used in these studies could not exclude the production of heteroduplex PCR artefacts arising from the simultaneous amplification of several closely related target sequences (Zylstra et al., 1998). Nor did their PCR primers allow any discrimination between the EG95 gene and other eg95 gene family members.
Table 1. Summary of EG95 vaccine trial results against an experimental challenge infection with *E. granulosus*.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cysts in individual sheep</th>
<th>Mean</th>
<th>Protection * %</th>
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<tbody>
<tr>
<td><strong>Trial 1</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Controls</td>
<td>V** 85 49 39 11 0</td>
<td>36.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NV 0 2 0 0</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>EG95 Vaccinated</td>
<td>V 0 0 0 0 0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>NV 1 0 0 0 0</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td><strong>Trial 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>V 16 9 9 2 2 2 2 1 1 0</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NV 25 14 8 12 6 4 45 7 4</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>EG95 Vaccinated</td>
<td>V 1 1 0 0 0 0 0 0 0 0</td>
<td>0.2</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>NV 4 1 4 3 2 1 0 0 0 0</td>
<td>1.5</td>
<td></td>
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<tr>
<td><strong>Trial 3</strong></td>
<td></td>
<td></td>
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<tr>
<td>Controls</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>NV 0 1 1 1 5 3 1 0 9 0</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>EG95 Vaccinated</td>
<td>V 1 0 0 0 0 0 0 0 0 0</td>
<td>0.1</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>NV 2 1 1 4 0 0 0 0 0 0</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td><strong>Trial 4</strong></td>
<td></td>
<td></td>
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<tr>
<td>Controls</td>
<td>T 165 40 30 15 10 8 7 3 2</td>
<td>28.9</td>
<td></td>
</tr>
<tr>
<td>EG95 Vaccinated</td>
<td>T 2 1 0 0 0 0 0 0 0 0</td>
<td>0.1</td>
<td>99</td>
</tr>
</tbody>
</table>

* Calculated on the number of viable cysts expressed as a percentage reduction in the mean number of cysts in vaccinated sheep compared with the mean number in control animals. ** V, viable cysts; NV, non-viable cysts; T, total cysts. Sheep were vaccinated with 50 µg protein plus 1 mg Quil A twice, one month apart, and challenged with *E. granulosus* eggs from parasites experimentally maintained in sheep and dogs in New Zealand (Trial 1), from a naturally infected Australian dingo (dingo/wallaby cycle; Trial 2), from a naturally infected Argentinian farm dog (dog/sheep cycle; Trial 3) and from a naturally-infected Chilian dog (dog/sheep cycle; Trial 4). Levels of protection were assessed 12-14 months after experimental infection (data from Lightowlers *et al.*, 1996a and 1999) except Trial 4, Drs Luis Rubilar and David Heath, unpublished observations.

Fig. 1. Schematic representation of the EG95 family of genes from a G1 genotype isolate (the same isolate from which the EG95 mRNA was originally isolated). Seven genes were identified in the genome, all of which are expressed except the pseudogene EG95-7 (after Chow *et al.*, 2001).
The gene found to encode the EG95 vaccine protein corresponds to the expressed product of the gene designated eg95-1 (Chow et al., 2001). However three other genes (eg95-2, eg95-3, eg95-4) express a protein identical to EG95 in the oncosphere of E. granulosus. Hence, the vaccine based on the expressed product of one gene would target the product of at least four genes. Two other genes in the EG95 gene family are also expressed in oncospheres. The proteins expressed by these genes are significantly different to EG95 and preliminary evidence suggests that these proteins are not host-protective (Heath, Gauci and Lightowlers, unpublished observations). Gene-specific investigations of genetic variability in E. granulosus with respect to the eg95-1 gene have begun (Chow, Gauci, Cowman and Lightowlers, unpublished observations) and, to date, no variation has been found in the predicted protein encoded by EG96 in any G1 genotype parasite examined to date. However significant variation has been found with G6/G7 genotype parasites. The potential impact this may have on the effectiveness of the EG95 vaccine for G6/G7 parasites remains to be determined.

Host-protective immune mechanisms induced by the EG95 vaccine

Two lines of evidence indicate that antibody and complement-mediated lysis of the invading oncosphere is a major host-protective mechanism induced by vaccination with the EG95 vaccine. Lambs born from EG95-vaccinated ewes are immune to E. granulosus challenge infection for the first few weeks of life (Heath et al., 2003) due to the presence of specific anti-EG95 antibodies received from their mothers via colostrum. Secondly, it is possible to demonstrate the killing of E. granulosus oncospheres by antibody raised against EG95 together with complement (Heath and Lawrence, 1981; Heath et al., 1981; Heath et al., 1994; Woollard et al., 1998; 1999). Oncosphere killing can be demonstrated by maintaining hatched and activated oncospheres in culture media together with heat-inactivated test sera and a source of complement. Sera from animals vaccinated with EG95 cause the death of oncospheres in the cultures whereas the presence of sera from control sheep do not prevent the survival and development of the cultured parasites. While this information does not exclude other host-protective immune mechanisms, it does indicate that specific antibody alone is sufficient to account for the host-protective effects of the EG95 vaccine.

Practical application of the EG95 vaccine

Heath et al. (2003) outlined the most effective procedure for use of the EG95 vaccine to prevent E. granulosus infection in livestock animals. Two immunisations are recommended in young animals, one month apart. The number of times each year that vaccinations would be given would depend on the local animal husbandry conditions. Where stock were borne over a single breeding season, only one vaccination period would be required per year (ie one vaccination period in which two vaccinations are given one month apart). In areas where stock are borne during two breeding seasons, two vaccination periods would be required because of the necessity to vaccinate young animals and ensure that they remain susceptible to infection for the shortest period practicable. Optimal levels of protection would be achieved if each of the young received both a primary as well as a booster immunisation within a short period of birth. However significantly enhanced protection could be expected if stock received a booster immunisation six-twelve months after the primary injections. Vaccine safety trials have been carried out in New Zealand, including investigations of safety in pregnant sheep and cattle. No adverse effects have been observed. Immunity persists after two injections for at least 12 months and longer term immunity can be achieved following a booster immunization given 6 or 12 months after the first two injections. Significant levels of protection can be transferred to lambs and calves with specific colostral antibody. In two large scale field trials in the Provinces of Qinghai and Xinjiang of China no adverse effects have been noted in the vaccinated animals and the vaccine has been highly effective in reducing naturally-acquired E. granulosus infection.

Preliminary investigations have indicated that the hydatid vaccine can be successfully delivered as a single immunisation mixed with multi-component clostridial vaccines, or mixed with an injectable anthelmintic. Incorporation of the hydatid vaccine as a component of other vaccines would provide an economic incentive to livestock owners to use such a vaccine while at the same time reducing the transmission of a medically important pathogen.

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