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# Vaccination trials in Australia and Argentina confirm the effectiveness of the EG95 hydatid vaccine in sheep

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**Abstract**

Experimental vaccine trials against hydatid disease have been undertaken in sheep using the EG95 recombinant vaccine. Challenge infection was with viable *Echinococcus granulosus* eggs obtained from a New Zealand isolate (dog/sheep cycle), an Australian isolate (dingo/wallaby cycle) and an Argentine isolate (dog/sheep cycle). Vaccination with EG95 conferred a high degree of protection against challenge with all three parasite isolates (protection range 96–100%). Taken together, the trials demonstrated that 86% of vaccinated sheep were completely free of viable hydatid cysts when examined approximately 1 year after challenge infection. Vaccination reduced the number of viable cysts by 99.3% compared with unvaccinated controls. These results suggest that the EG95 vaccine could have wide applicability as a new tool for use in hydatid control campaigns. 

*Keywords:* *Echinococcus granulosus*; EG95; Hydatid; Recombinant antigen; Sheep; Vaccine

Infection in humans with the cestode parasite *Echinococcus granulosus* causes cystic or unilocular hydatid disease. The infection occurs endemically in many countries of the world and is particularly prevalent in the Mediterranean littoral, parts of Africa, Russia, China, Australia and the southern zone of South America. Control of transmission of the disease relies on education of the public to prevent access of dogs, the parasite's definitive host, to the offal of infected intermediate hosts, and the regular treatment of dogs with a cestocidal anthelmintic. Control campaigns based on those methods can be successful; however, they typically require intensive effort over several years. Any breakdown in infection control in dogs may lead to substantial numbers of new infections in intermediate hosts, providing a continuing source of

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infection for transmission of the parasite. Although many species of domestic livestock and herbivorous wildlife species are potential hosts for *E. granulosus*, sheep play a major role in transmission of the parasite globally.

A recombinant antigen vaccine has been developed for use as an additional tool in hydatid control campaigns [1]. Experimental trials in New Zealand using the EG95 antigen achieved 96–97% protection against a challenge infection with *E. granulosus* eggs in sheep. The parasites used for the challenge infection in the trials described previously had been maintained for approximately 20 years at the Wallaceville Animal Research Centre, Upper Hutt, by serial passage through sheep and dogs. The original isolates of the parasite were obtained from naturally infected sheep. *Echinococcus granulosus* is known to occur as a complex of strains, differing in such areas as host preference, morphological, biochemical and molecular characteristics. A thorough molecular analysis of variation in the EG95 protein/gene is in progress. However, in order to obtain rapid information concerning the general applicability of the EG95 vaccine for use against *E. granulosus*, vaccine trials have been completed against experimental challenge infection in sheep with *E. granulosus* occurring endemically in Australia and Argentina.

Three experimental vaccine trials have been undertaken. Initially, a vaccine trial was undertaken in Australia (Trial 1) in Dorset/Merino cross lambs against challenge infection using *E. granulosus* eggs obtained from the Wallaceville Animal Research Centre in New Zealand. EG95 vaccine protein was expressed in *Escherichia coli* transformed with pGEX-3-EX containing the EG95 cDNA, as described in Lightowlers et al. [1]. The glutathione *S*-transferase fusion protein was affinity purified from *E. coli* lysate [2], the protein content estimated by Coomassie dye binding and purity determined in SDS–PAGE. After filtration through an 0.22-$\mu$m filter, the vaccine was adjusted to 25 $\mu g\,ml^{-1}$ with sterile PBS including a final concentration of 0.5 $mg\,ml^{-1}$ Quil A (Superfos). Lambs, 4–6 months of age of mixed sex, were vaccinated s.c. in the neck with a 2-ml dose of vaccine (i.e. 50 $\mu$g EG95 plus 1 mg Quil A) on two occasions 4 weeks apart. Control lambs were unvaccinated (previous trials [1] found that placebo GST vaccination had no effect on susceptibility to *E. granulosus*). Serum samples were obtained at intervals during the trial. Two weeks after the second vaccination, the lambs received a challenge infection with 1000 viable *E. granulosus* eggs prepared as described in Ref. [3]. Eggs were suspended in 10-ml water and injected into the rumen via a 16G 10-cm syringe needle fitted with a disposable three-way stopcock (Discufix, Braun). Prior to injection of eggs, a small amount of water was injected into the rumen via a second syringe connected to the stopcock and the fluid immediately withdrawn to ensure that rumen contents were visible, indicating correct placement of the needle. After injection of the eggs the needle was flushed with approximately 20-ml water via a second syringe also fitted to the stopcock. This method ensured the delivery of the same dose of infective eggs into the rumen of each animal and prevented any possibility of contamination of the operators with *E. granulosus* eggs. Approximately 12 months after the experimental infection, the sheep were euthanased and examined for hydatid cysts. The carcasses were dressed and examined superficially for the presence of hydatid cysts. The heart and kidneys were sliced and the omentum and spleen also examined. The liver and lungs were examined extensively. The liver was sliced at intervals of approximately 3 mm. The lungs were sliced at intervals of approximately 6–8 mm and palpated. Hydatid cysts were examined and deemed viable if they contained a cavity and clear cyst fluid. Cysts comprising only fibrotic, caseous or calcified material were recorded as non viable. Samples of cystic material were fixed and examined histologically in situations where cyst viability could not be established macroscopically.

Enzyme-linked immunosorbent assays were performed on sera from sheep in Trial 1 using an EG95–maltose binding protein (EG95–MBP) as antigen, as described by Woollard et al. [4]. Prominent primary and secondary immune responses were induced by the two immunisations and the sheep remained seropositive for the

EG95 component of the vaccine for more than 1 year (data not shown).

In Trial 1 the EG95 vaccine induced complete protection against the challenge infection in four of the five vaccinated animals (Table 1). In this trial, the same source of challenge parasites (from New Zealand) was used as in previously published trials [1], but in a different breed of sheep and carried out in a different laboratory (Werribee).

Trial 2 was set up using a similar protocol to Trial 1, but used a different source of parasites for the challenge infection. Mature *E. granulosus* tapeworms were obtained from a naturally infected dingo captured in the Snowy Mountains National Park, Australia. *Echinococcus granulosus* tapeworms were recovered from the small intestine of the dingo and mature tapeworms were selected under a dissecting microscope on a dark background using fine forceps. The tapeworms were stored at 4°C for 10 days in 0.85% saline containing 1000-units penicillin, 1-mg streptomycin and 100-units nystatin per millilitre. Immediately prior to the challenge infection the worms were diced with fine scissors and the eggs filtered through a 100-$\mu$m sieve. Eggs were counted using a modified Fuch's Rosenthal chamber (B.S. 748, Weber Scientific International) and made up to 40 eggs $ml^{-1}$ in water. The challenge dose comprised 400 mature eggs. Sheep were merino breed and approximately 7 months old at the beginning of the trial. Necropsy and assessment of the level of protection was performed 12 months after the challenge infection. The trial results are shown in Table 1.

Although the level of infection in control animals was low, eight of the 10 vaccinated sheep had no viable hydatid cysts compared with the presence of viable cysts in all except one of the control sheep. Based on the number of viable cysts, the vaccine was 96% effective.

Trial 3 was undertaken in Sarmiento, Chubut Province, Argentina. The protocol was essentially as described above. EG95 antigen was prepared in Australia and freeze dried in two aliquots sufficient for the primary and secondary immunis-

Table 1
Numbers of hydatid cysts and levels of protection achieved in sheep vaccination trials using the EG95 recombinant vaccine[a]

| Group | | No. of cysts in individual sheep[b] | | | | | | | | | | Mean | Protection[c] (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trial 1 | | | | | | | | | | | | | |
| Controls | V | 85 | 49 | 39 | 11 | 0 | | | | | | 36.8 | |
| | NV | 0 | 2 | 0 | 0 | 0 | | | | | | 0.4 | |
| EG95 vaccinated | V | 0 | 0 | 0 | 0 | 0 | | | | | | 0 | 100 |
| | NV | 1 | 0 | 0 | 0 | 0 | | | | | | 0.2 | |
| Trial 2 | | | | | | | | | | | | | |
| Controls | V | 16 | 9 | 9 | 2 | 2 | 2 | 1 | 1 | 0 | | 4.7 | |
| | NV | 25 | 14 | 8 | 12 | 6 | 4 | 45 | 7 | 4 | | 13.9 | |
| EG95 vaccinated | V | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2 | 96 |
| | NV | 4 | 1 | 4 | 3 | 2 | 1 | 0 | 0 | 0 | 0 | 1.5 | |
| Trial 3 | | | | | | | | | | | | | |
| Controls | V | 64 | 62 | 51 | 23 | 11 | 7 | 4 | 4 | 3 | 2 | 23.1 | |
| | NV | 0 | 1 | 1 | 1 | 5 | 3 | 1 | 0 | 9 | 0 | 2.1 | |
| EG95 vaccinated | V | 1 | 0 | 0 | 0 | 0 | 0 | 0 | | | | 0.1 | 99 |
| | NV | 2 | 1 | 1 | 4 | 0 | 0 | 0 | | | | 1.1 | |

[a] Sheep were vaccinated with 50-$\mu$g protein plus 1-mg Quil A twice, 1 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) and from a naturally infected Argentine farm dog (dog/sheep cycle). Levels of protection were assessed 12–14 months after experimental infection.

[b] V, viable cysts; NV, non-viable cysts.

[c] 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.

ations. Antigen was lyophilised together with Quil A in a ratio of 1-mg Quil A per 50-$\mu$g EG95. Sheep were of merino breed and approximately 7 months old at the beginning of the trial. Mature *E. granulosus* worms for the challenge infection were obtained from a naturally infected farm dog in the Chubut Province of Argentina. Challenge infection was per os and comprised four complete, mature *E. granulosus* tapeworms (containing an estimated 1200 eggs) given 9 weeks after the second immunisation. Necropsy was performed 14 months after the challenge infection. The results of Trial 3 are shown in Table 1. One of the vaccinated animals had a single viable cyst, the remainder had no infection. All 10 control sheep had viable hydatid cysts at post mortem.

No information is available as yet concerning genetic variability in the gene encoding EG95 among different isolates or strains of *E. granulosus*. All three of the isolates used in the trials discussed here would be expected to belong to the GI group as defined by Bowles et al. [5]. This appears to be the major genetic group within *E. granulosus*. The effectiveness of the EG95 vaccine against *E. granulosus* from Australia, Argentina and New Zealand suggests that EG95 is likely to have broad applicability as a vaccine against cystic hydatid disease. Preliminary reports from extensive EG95 trials which have been conducted in China [6] suggest that the vaccine is also effective against *E. granulosus* of Chinese origin.

Taken together, the results of the three vaccine trials described here find that 86% of vaccinated sheep were free of any viable hydatid cysts when examined approximately 1 year after challenge infection. The total number of viable cysts was reduced by 99.3% in vaccinated sheep. Nevertheless, the vaccine does not completely prevent the possibility of viable hydatid cysts developing in vaccinated sheep. Three of the 22 vaccinated animals in these trials developed a single, viable cyst. One possible explanation for the occurrence of these cysts in vaccinated sheep is that they represent a small proportion of the parasite population which express immunological variants of the EG95 protein which are non-susceptible to the vaccine. Such a possibility could have important consequences for the widespread use of the vaccine. Preliminary investigations indicate that a single *E. granulosus* parasite contains six or more genes which may encode proteins which are slightly different to EG95 (C. Chow, unpublished data). Characterisation of these genes, and their encoded proteins, will allow the genetic variability of the EG95 protein to be determined in different *E. granulosus* isolates.

The high level of protection achieved in the three vaccine trials described here using *E. granulosus* parasites from Australia, Argentina and New Zealand suggest that the EG95 vaccine would have wide applicability as an effective control measure against transmission of cystic hydatid disease.

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