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Maternal antibody parameters of cattle and calves receiving EG95 vaccine to protect against *Echinococcus granulosus*

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ABSTRACT

Cattle may act as hosts for the transmission of the cestode parasite *Echinococcus granulosus* and play a role in transmission of the parasite leading to human cystic echinococcosis (CE). The recombinant EG95 vaccine has been shown to be able to protect cattle and other intermediate host species against CE. Ideally the immunisation of bovines against *E. granulosus*, using EG95 vaccine, should occur early in life so as to provide maximum protection against the establishment of hydatid cysts. Maternally derived antibody from vaccinated cows may provide some protection for the neonate, but may also interfere with the active response to vaccination. Experiments were undertaken to determine the optimal regime for protection of young cattle against CE. One group of pregnant cattle received 2 vaccinations of EG95 antigen + Quil A adjuvant two months and one month prior to calving. The control group of pregnant cattle were not vaccinated. Calves were either challenged with *E. granulosus eggs* at 4, 9, 13 or 17 weeks post-birth or were given their first vaccination at 8, 12 or 16 weeks post-birth. Sera obtained at regular intervals were tested by ELISA to assess the immunological response. All calves were experimentally challenged with *E. granulosus* eggs and subsequent necropsy confirmed the levels of protection. Maternal antibody was shown to protect calves to some extent for at least 17 weeks. Calves from vaccinated cows responded well serologically if the first vaccination was given at 8 or 12 weeks, but full protection against a challenge infection was achieved only if the first vaccination was delayed until 16 weeks after birth. Calves from non-vaccinated cattle also were not fully protected if the first vaccination was at 8 or 12 weeks, but were fully protected if the first vaccination was given when they were 16 weeks old. This suggests that immunological maturity is not acquired in calves until 4 or 5 months of age. No safety problems were observed following two vaccinations of 40 pregnant cows or 30 suckling calves.

1. Introduction

A variety of animals may act as intermediate hosts for *Echinococcus granulosus* and contribute to transmission of the parasite leading to human infections with cystic echinococcosis (CE). Cattle and other bovines may be infected with *E. granulosus*, including the G1 genotype that is known to cause the majority of human infections [1–5]. The EG95 vaccine has been developed which is capable of protecting various species of intermediate host against CE, including bovine hosts [6–9]. In implementing the EG95 vaccine for control of CE in bovines, it would be valuable to be able to protect young animals from infection so as to minimise the possibility that infection was acquired prior to vaccination. The experiments described in this paper sought to determine the optimum regime for providing protection to young calves against CE.

Antibodies in colostrum can be absorbed across the new-born calf intestine for only 2 days after birth [10] and are known to have an impact on vaccine efficacy in young animals [11]. Maternally derived antibodies (MDA) against rinderpest or bovine virus diarrhoea (BVD) virus can persist in calves for more than 9 months [11,12]. Maternally derived antibodies can interfere completely with live attenuated viral vaccines, where active immunity is generated by proliferation of the virus [13]. Calves were able to seroconvert to BVD vaccine at 3 months of age, but responded best after MDA had disappeared at 6.5 months of age. MDA was shown to block antibody responses to BVD virus but did not block T cell responses [14]. The immune system of calves does not normally reach full maturity until 5–6 months of age. This does not mean that a young calf cannot respond to antigens, but the response will be weaker [15]. In mature animals over 7–8 years the immune response declines with age.

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Table 1
Number of hydatid cysts in individual calves following a challenge infection with *E. granulosus* eggs. Calves were born from either non-vaccinated or EG95 vaccinated cows ($N = 5$ in each group).

Group number	Cow treatment	Calf age at challenge infection (weeks)	Number of hydatid cysts in individual calves	Mean	Protection (%)[a]
1	V	4	0, 0, 0, 1, 12	2.6	Nil
2	NV	4	0, 0, 3, 3, 6	2.4	
3	V	9	0, 0, 1, 3, 6	2.0	88[a]
4	NV	9	12, 13, 14, 14, 30	16.6	
5	V	13	0, 1, 3, 10, 26	8.0	34
6	NV	13	0, 4, 5, 20, 32	12.2	
7	V	17	0, 0, 2, 3, 3	1.6	94[a]
8	NV	17	7, 23, 29, 30, 50	27.8	

[a] Statistically significant reduction in the number of cysts in vaccinates compared with non vaccinated controls ($P < 0.05$; Mann–Whitney-*U* test).

Some information is available concerning immunity in calves to *Taenia saginata* following vaccination with native or recombinant antigens. A partially effective passive transfer of immunity from cows immunised with *T. saginata* oncosphere antigens has been demonstrated [16,17]. Rickard et al. [17] showed that strong immunity was generated in calves if vaccinated when 8–10 weeks old with oncosphere secretory antigens obtained during in vitro culture. A field trial using this antigen preparation showed that vaccinating pregnant cows reduced the number of *T. saginata* cysts found in calves. Also, some calves were vaccinated at 1.5–4 months old. The most effective vaccination regime was pre-calving vaccination of the cow followed by vaccination of the calf. This regime reduced natural challenge infection by 78% [18]. When calves were vaccinated with recombinant antigens [19], 99.8% protection was induced against a challenge infection with *T. saginata* eggs. It was recognised that the parameters of MDA stimulation in the cow and efficiency of protecting the calf, immunisation of the calf in the presence of MDA, and longevity of immunity, remained to be determined.

To date, no information is available regarding the transfer of maternal antibody to EG95 from cattle and whether it provided protection to the calves against *E. granulosus* infection and also there was no information regarding vaccination in the presence of MDA. Here we detail the results of vaccine trials designed to determine the length of time that maternally derived antibody provided protection against *E. granulosus* infection in calves born to vaccinated cows and to establish an appropriate time, post-birth, that calves may be vaccinated with the EG95 vaccine without MDA interfering with the response.

2. Materials and methods

2.1. Ethical approval

All work described here: dog infections with *E. granulosus*; necropsy of sheep and cattle; vaccination of cattle; infection with *E. granulosus* eggs; was approved by the AgResearch, Wallaceville Animal Research Centre Animal Ethics Committee.

2.2. Animals

From 100 pregnant cows (Friesian–Hereford cross, mated to a Hereford), 40 received 2 vaccinations of EG95 two months and one month prior to calving. Groups of calves from the vaccinated and non vaccinated cows were challenged with *E. granulosus* eggs at various times after birth in order to assess passive transfer of protection with MDA. These groups of calves were challenged at 4, 9, 13 or 17 weeks of age as indicated in Table 1. The remaining calves were used to determine the effects, if any, of the presence of MDA on responses to active immunization with EG95, and their immunity to *E. granulosus* infection. Immunised calves received 2 injections with EG95, one month apart, with the first immunization being given at 8, 12 or 16 weeks of age, as indicated in Table 2. Non vaccinated calves born to non vaccinated cows acted as controls. The calves were challenged 4 weeks after the second vaccination. All calves were necropsied nine months after the challenge infection with *E. granulosus* eggs. At the necropsy, livers were sliced at 4 mm intervals and lungs at 6 mm intervals. All slices were palpated to detect any cysts that were not obvious on the surface. All lesions detected were cut open to prove that they were living *E. granulosus* cysts with a fluid-filled central cavity. Cyst internal diameters ranged from 1 to 3 mm in the liver and from 2 to 5 mm in the lungs. The protective effect of the vaccine was calculated by comparing the mean number of cysts found in the 5 animals of a control group with the mean number of cysts in the treated group corresponding to the control group. This can be observed in Tables 1 and 2. No protoscoleces were observed in these juvenile 9-month-old cysts.

2.3. Animal handling and serum sampling

Calves were ear-tagged immediately after birth. Once all of the calves had been born, they were allocated into the experimental groups as shown in Tables 1 and 2 and treated accordingly. Vaccinated cows were kept in separate paddocks from control cows before calving commenced to ensure there was no cross-suckling. A blood sample for serum collection was obtained from each calf at approximately 5 days of age and subsequently 2 weeks later. Blood samples were also obtained at each vaccination and challenge, using 10 ml Vacutainer SST tubes and 18 g needle. At the time of challenge, the calves requiring a challenge and their corresponding mothers were transferred to the quarantine area. All calves were weaned at 16 weeks of age.

2.4. Vaccine formulation

EG95 vaccine was prepared as solubilised inclusion bodies as described by Manderson et al. [20] (Lot # HYD/005) and sterile filtered through a 0.2 μm membrane. Each vaccine dose contained 250 μg EG95 and 5 mg of the adjuvant Quil A (Lot # L77-172; Superfos-Biosector, Denmark). Vaccine was stored in 50 mL sterile bottles with rubber cap at 4 °C until required. The dose was 5 mL/vaccination. All injections were given subcutaneously in the neck region using an 18 gauge needle of 2 cm length.

2.5. ELISA for detection of anti-EG95 antibody

The EG95 antigen used for ELISA was prepared by expressing Eg95-6HIS in *Escherichia coli*, and purifying the construct on Ni-NTA resin (Qiagen). The procedure was as described by [8], except that rabbit anti-bovine IgG (h + l) HRP conjugate (Sigma–Aldrich) 1:4000 was used.