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# TOWARDS A COMMERCIAL VACCINE AGAINST *HAEMONCHUS CONTORTUS* - A FIELD TRIAL IN WESTERN AUSTRALIA

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## Introduction

*Haemonchus contortus* is the most important nematode parasite of small ruminants on a global basis, both as a major cause of mortalities and due to the costs of control. The need for frequent anthelmintic treatment to prevent animal deaths has led to the widespread development of anthelmintic resistance in *H.contortus* in Australia (Besier and Love 2003) and more widely (Kaplan 2004), which has severely limited the effectiveness of control measures. Alternative approaches to anthelmintics have been sought for many years, but these investigations are yet to yield significant advances in new technologies.

The potential to produce an effective vaccine against *H.contortus* has been evident for many years, using “hidden antigens” extracted from worm intestinal membranes. Significant reductions in worm burden and worm egg counts of vaccinated sheep have been demonstrated with this approach in numerous pen experiments in the UK, commencing over two decades ago (eg, Munn et al 1993, Smith 1993). However, although the immunological basis has since been extensively investigated and protective antigens characterised (Knox et al 2003), it has not proved possible to reproduce the protective effects in sheep when the specific proteins were produced in recombinant systems (reviewed by Newton and Meeusen 2003, and Cachat et al 2010). Prospects for the mass-production of an *H.contortus* vaccine by this means appear poor at present.

While vaccine production by recombinant technology has been unsuccessful, trials have confirmed the efficacy in the field of a vaccine produced at the Moredun Research Institute in Edinburgh from “native antigens”, extracted from adult *H.contortus* in sheep. Vaccination with a combination of antigens in a trial in South Africa showed worm egg count reductions of over 80% with simultaneous reductions in anaemia and sheep deaths (Smith et al 2001); computer modelling studies suggest that over a grazing season, the flock effect of pasture contamination of a similar or lower degree will largely prevent the development of significant *H.contortus* burdens (Barnes et al 1995). More recently, a field trial in New South Wales of a *H.contortus* vaccine with similar antigens showed results at least as good (Le Jambre et al 2008), despite clinical haemonchosis in untreated control group lambs. Both trials confirmed that repeated vaccination at intervals of some weeks was necessary to maintain season-long protection and that as with pen trials, plasma antibody levels followed parasitological and haematological indices relatively closely.

Although effective in field trials, commercial production of this native vaccine was not considered feasible due to the apparent quantity of antigen required. However, a review of this requirement led to pen trials that demonstrated that only a relatively low

dose of antigen was necessary to provoke a protective response (W.D.Smith, pers com). It was therefore postulated that the material gained from a single infected sheep could provide sufficient vaccine to protect some thousands of animals, and that harvesting *H.contortus* on a commercial basis could be economically feasible. Subsequent investigations using a specialised worm harvesting machine have confirmed that large quantities of *H.contortus* can be recovered rapidly at slaughter from artificially-infected lambs raised under commercial conditions, without pathogenic effects, and relatively large batches of a prototype vaccine have been produced. A cooperative venture between the Moredun Research Institute and Department of Agriculture and Food WA is now investigating the development of a *H.contortus* vaccine for commercial markets.

The trial in Western Australia reported here was intended to confirm the effectiveness under field conditions of a vaccine produced at Albany by Moredun scientists, using worm material recovered from sheep artificially infected with *H.contortus* larvae before slaughter at a local abattoir. Specifically, the trial compared four different vaccine antigen doses and two administration schedules, to indicate a regime which would prevent the clinical effects of haemonchosis (evident as high worm egg counts or anaemia) over a larval challenge period of at least 3 months.

**Materials and methods**

The trial was conducted from October 2010 to January 2011 on the Mt Barker Research Station (approx. 50 km North West of Albany), on a mostly perennial-pasture paddock that dried off in late December. The trial sheep (5 month-old Merino-Poll Dorset cross lambs) were drenched to remove all worms prior to entry to the trial paddock (previously contaminated by *H.contortus*-infected sheep), on the day of the first vaccination. They were run as a single flock in 9 treatment groups: an untreated control (n =20); and 8 vaccinated groups (n = 12): 4 “early third vaccination (V3)” groups given *H.contortus* antigen doses of 2, 5, 10 or 50 micrograms (μg); and four “late V3” groups given the same vaccine doses. The vaccination schedule was: V1 (late September); V2 (3 weeks later); V3, either at 6 weeks (early) or 8 weeks (late) after V2; V4 (6 weeks later, early V3 only). In addition, 3 groups of worm-naïve “tracer” lambs were run with the trial flock for 2 week periods at 4-weekly intervals to assess the intake of *H.contortus* larvae from pasture.

Over the course of the trial the lambs were orally dosed with *H.contortus* infective larvae, at twice-weekly intervals for approximately half the trial period, then at weekly intervals until natural infection increased. Faecal worm egg counts and the blood status (packed cell volume, PCV) of all lambs were measured fortnightly once infections were established, and weights and body condition score monitored. ELISA assays for serum antibodies against the vaccine antigens were also conducted. Anthelmintic treatment was given to all sheep at the start of the trial start and thereafter to any individual with a PCV less than 20%.

**Results**

The trial sheep were exposed to relatively heavy *H.contortus* larval challenge, indicated by tracer sheep worm counts to be initially mostly from artificial infections, and later also by natural intake from pasture. *H.contortus* egg counts in the control group reached a group mean maximum of 7000 eggs per gram (epg) by the last week of the trial (Figure 1), with mean counts over 3000 epg for the last 8 weeks. These figures are an underestimate, as 7 of the 20 control sheep were drenched at various times (due to low PCVs), with the counts of some of these animals exceeding 20,000 epg.

In contrast, the mean counts from V2 onward of the groups vaccinated with 10 or 5 µg of antigen at “early V3” were reduced by a mean of 87% compared to control counts; the reduction increased to 96% when one “non-responder” sheep in the 5 µg group is removed from the calculations. (These animals fail to develop an effective immune response, as indicated by atypically high egg counts and low antibody levels; see Figure 1). However, the same antigen doses (5 or 10 µg) at “late V3” gave less significant reductions in egg counts (76% compared to control sheep counts, adjusted for one non-responder); for the lowest doses (2 µg) the reduction was 63% regardless of the time of administration. As the protection levels from “early V3” 5, 10 or 50 µg or antigen doses were not statistically different from each other when measured over equivalent periods after vaccination, it is concluded that 5 µg of antigen provided optimal protection for the minimum dose.

Of interest, a significant anthelmintic effect due to vaccination was evident following “early V3” treatments, with a reduction of more than 90%, although there was only a 70% reduction for the “late V3” groups.

The comparative *H.contortus* egg count differences were not as dramatically reflected in the measures of anaemia, as mean PCVs of both control and vaccinated groups declined by to a similar degree over the trial period (36 to 29%). However, the final figures for the vaccine dose groups with lowest worm egg counts (50 µg, and “early V3” 5 and 10 µg) were 2-3 % PCV higher than those of the control group. Further, 7 of the 20 control group sheep required remedial drenching compared with one of the 12 sheep in each of these vaccinated groups. All sheep gained weight over the course of the trial period (a mean of 14.3 kg), but the control sheep were between 1 and 2 kg lighter than the groups showing greatest vaccine effect.

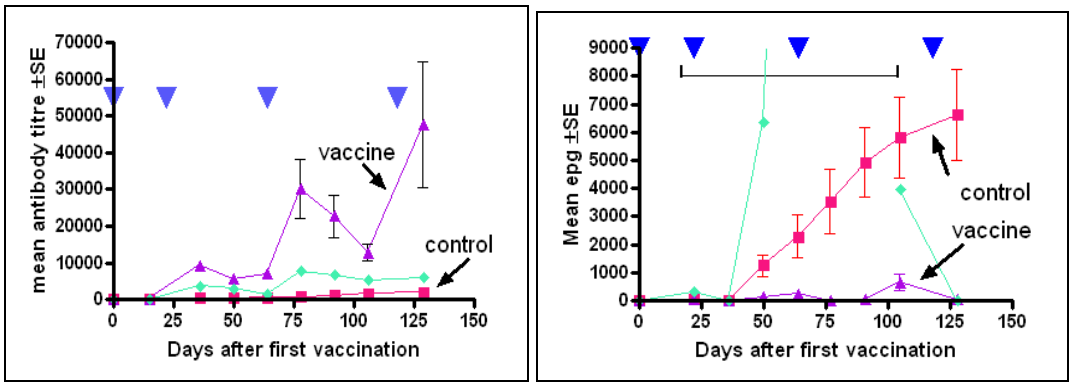


**Figure 1:** Group means (± sem) for serum antibody titres (left) and *H.contortus* egg counts (right) for Control and Vaccinated (“early V3”, 5 µg) groups. Inverted triangles indicate dates of vaccination. Diamond symbols indicate one non-responder sheep.

**Discussion**

This trial confirmed that as little as 5µg of *H.contortus* “native antigen” vaccine was sufficient to provide a high level of protection over a prolonged period of active larval intake, when given at 6-weekly intervals after 2 priming vaccinations.

Our studies have shown that several grams of adult Haemonchus can be recovered cost effectively from a donor sheep without compromising its carcase value, health or growth rate. Due to the small quantity of antigen needed, each donor animal can yield a few thousand doses of vaccine, and the antigen can therefore be produced at a sufficiently low cost for the vaccine to have commercial potential.

The reductions in *H.contortus* egg counts in sheep given vaccine doses of 5 µg and above exceeded 85% over the course of the trial, with no deaths or significant anaemia despite the need for remedial treatment of some 30% of the unvaccinated control sheep. The protective response included both the reduced development of worm burdens from larval intake and a significant anthelmintic response, seen as a sharp fall in worm egg count after vaccination. The combined effect is well above that indicated by computer simulation modelling as required to provide both immediate protection and a longer-term epidemiological benefit (Barnes et al 1995).

The results indicate that vaccination will allow a considerable reduction in anthelmintic treatment over periods of haemonchosis risk, with a consequent reduction in the development of anthelmintic resistance. Although a small number of sheep appeared to be “non-responders”, it is likely that such immunologically-impaired animals are always at particular risk from infective agents. However, the epidemiological effect of vaccination will reduce larval intake from pasture and hence the relative risk of excessive *H.contortus* burdens by these individuals.

Further investigations are required in different environments and under different rates of *H.contortus* challenge, but the results from this trial, and from concurrent work in other countries, suggest a realistic commercial potential for a vaccine based on native *H.contortus* antigen.

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