



Vaccination against *Haemonchus contortus*: Performance of native parasite gut membrane glycoproteins in Merino lambs grazing contaminated pasture

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Abstract

In a replicated trial, parasitological and antibody responses of grazing weaner Merino sheep were assessed following vaccination with gut membrane proteins prepared from adult worms of the gastrointestinal nematode, *Haemonchus contortus*. Each vaccinated animal received 100 µg native H11 and 100 µg native H-gal-GP combined together in 5 mg Quil A administered intramuscularly on days 0, 34, 80 and 127. Control animals received 5 mg Quil A alone on the same days. Vaccinated and unvaccinated control animals grazed pastures contaminated with the parasite from day 34 of the trial, and levels of parasitism were monitored by worm-egg counts (WECs) in faeces and packed cell volumes (PCVs) in blood. The level of larval contamination on pasture was estimated from the worm counts of tracer sheep introduced monthly to the paddocks. WECs and anaemia were significantly reduced in vaccinated animals, and, in contrast to vaccinates, all control sheep required salvage treatment with anthelmintic. By the last 2 months of the trial, pastures grazed by vaccinated animals had significantly lower contamination with *H. contortus* larvae. Vaccinated animals had high levels of vaccine antigen-specific IgG1 and IgG2 antibodies in plasma, whereas those responses in the control sheep were very low. IgG1 titres in the vaccinated group, but not IgG2 titres, were inversely correlated with worm-egg counts. The levels of systemic IgA and IgE remained low but increased in both groups towards the end of the experiment most probably from exposure to the natural infection from pasture. The results showed that H11 and H-gal-GP behaved like “hidden” antigens producing high levels of protection that were probably mediated through mechanisms involving antibodies, and in particular, IgG1. It was concluded that if similar protective effects could be obtained with recombinant versions of the proteins present in either H11 or H-gal-GP, then the prospects for a commercial *Haemonchus* vaccine were real.

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1. Introduction

The nematode *Haemonchus contortus* is probably the single most economically important sheep parasite

in the world. Traditionally, control has relied almost entirely on the use of anthelmintic drugs, but strains of *H. contortus* resistant to one or more of these chemicals are now common (Waller, 1997; Besier and Love, 2003). Various alternative control methods are being pursued, one of which is vaccination.

Although a number of protective antigens have been described for *H. contortus*, two glycoprotein complexes known as H11 and H-gal-GP from intestinal cells of

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fourth stage and adult parasites are amongst the best studied and most effective (reviewed by Smith and Zarlenga, 2006; Newton and Meeusen, 2003; Knox et al., 2003). Most of the protection data for these antigens have been derived from vaccine challenge trials conducted with worm free sheep given single artificial challenge doses of larvae in pens, though two partially successful trials with adult grazing sheep have also been reported (Smith et al., 2001a,b; Kabagambe et al., 2000).

This paper describes the results of a vaccine trial using a combination of native H11 and H-gal-GP in Merino weaner lambs that grazed replicated paddocks contaminated with *H. contortus*. The primary purpose of the trial was to determine whether these gut membrane proteins had the potential to provide a useful level of *H. contortus* control in young sheep under conditions which closely resembled those encountered by commercial sheep farmers in a part of Australia where wool is an important commodity.

2. Materials and methods

2.1. Sheep

The animals used in this experiment were fine wool Merino lambs that came from the breeding flock at the Chiswick Research Station, CSIRO Livestock Industries, Armidale, NSW. This work was carried out with the approval of the local CSIRO animal ethics committee (McMaster Laboratory approval number 03/37).

2.2. Egg and worm counts

Worm-egg counts (WECs) were determined on animals using a modified McMaster method in which each egg counted represented 100 eggs g⁻¹ (epg) of faeces. Blood was taken from each sheep at the same time as faecal collection in order to estimate packed cell volumes (PCVs) and to conduct serology.

Tracer sheep were weaned into the animal house, drenched with levamisole, ivermectin and oxfendazole and kept under conditions which precluded further nematode infections until being moved to the experimental paddocks where they were allowed to graze for one fortnight. At the end of this period the tracers were returned to the animal house for a further fortnight. This allowed recently ingested worms to develop to at least immature adult stage and facilitated finding them amongst intestinal contents. When the sheep were slaughtered for worm counts their abomasum was

removed immediately and the contents were washed and scraped into two-litre graduated cylinders. After settling, the abomasal fluid was reduced to a volume of 600 ml. Three 30 ml sub-samples were frozen for counting and the remainder was discarded. Thus, the worm burdens in the abomasum are based on counts of three replicate 5% samples.

2.3. Vaccine preparation and administration

The peanut and ConA lectin binding fractions of detergent extracted membranes of adult *H. contortus* (42 g) were prepared exactly as described before (Smith et al., 2001a,b) using an isolate originally obtained from Wallangra (29°09'S, 150°53'E) near Armidale in 2003 (kept as frozen larvae at FD McMaster Laboratory). The approximate protein content of each fraction was determined with a spectrophotometer using the formula: concentration (mg/ml) = $(1.55 \times A_{280}) - (0.76 \times A_{260})$. Equal amounts of protein from each fraction were pooled and diluted with a QuilA (Brenntag, Denmark) phosphate buffered saline solution so that each lamb received a total of 100 µg of each fraction and 5 mg QuilA on each vaccine day. The vaccine was administered intramuscularly as a 2 ml dose, 1 ml being injected into each back leg.

2.4. ELISAs

The ELISA procedures used in this experiment were adapted from those described previously (Windon et al., 2002; Bendixsen et al., 2004) and were used to assess specific antibody responses to two distinct antigen preparations. The antigens used were the vaccine antigen (VA, as described above) and a soluble protein extract prepared from homogenized *H. contortus* infective larvae (SP3) as described by Emery et al. (1991). The antibody isotyping reagents used were anti-ovine immunoglobulin (total antibody) conjugated to horseradish peroxidase (HRP) (Chemicon, Melbourne, Australia), mouse anti-sheep IgG1, IgG2 and IgM (Beh, 1987, 1988), mouse anti-bovine IgA (VMRD Inc., Pullman, WA, USA) which cross-reacts with ovine IgA, and a monoclonal antibody raised in mice against an ovine chimeric IgE protein (Bendixsen et al., 2004).

Microtitre plates (96 well, Maxisorb, Nunc, Roskilde, Denmark) were coated with either 0.05 µg per well (0.5 µg ml⁻¹) of VA or 0.1 µg per well (1 µg ml⁻¹) of SP3 in coating buffer (pH 9.6) overnight at 4 °C. Following blocking with 0.1% (w/v) sodium casein (Sigma, Australia) in PBS for 2 h at RT, the plates were washed once with PBS containing 0.05%

Tween 20 (PBST). Plasma samples from each sheep were optimally diluted for each isotype assay and 100 μ l was serially diluted in PBST containing 1% (w/v) sodium casein and the plates incubated for 1 h at room temperature (RT). After four washes, isotype specific reagents (100 μ l) were added as required at optimal dilutions and incubated for 1 h at RT. For all assays except total Ig, 100 μ l sheep anti-mouse Ig conjugated to HRP (Chemicon, Melbourne, Australia), diluted 1:2000, was added to each well and incubated 1 h at RT. The plates were washed six times and 100 μ l tetramethyl benzidine (TMB) substrate (Bos et al., 1981) was added to each well. After 30 min at RT, 50 μ l 0.5 M H_2SO_4 was added to each well to stop chromophore production and the absorbance read at 450 nm using an Thermomax Microplate Reader (Molecular Devices, CA, USA). Individual titres were determined by calculating the midpoint, on a double log scale, of the straight line section of the curve using linear regression, and represent the inverse of the dilution at these points.

2.5. Statistical analysis

Egg counts and PCVs were analysed with Genstat using linear mixed models.

Egg counts were log transformed to model the large number of zero values present in the data. "Paddock" and "Sheep.Day" were fitted as random effects, assuming uniform covariance between times for each sheep, as in a split-plot design.

No transformation was found to be necessary with the PCV data to generate residuals consistent with the assumptions of the model. Plot and Sheep.Day were fitted as random effects, and the autocorrelative structure in day was modelled as an autoregressive, order 1 model.

Fishers exact test was used to detect differences between the vaccinated and control groups with respect to number of deaths and salvage treatments.

Differences in vaccine and control paddock infectivity were investigated with Student's *t*-test.

2.6. Design of the vaccine experiment

The vaccine field trial was conducted on the "Chiswick" research station (30°36'S, 151°32'E), using four adjacent 1 ha paddocks. These paddocks had not been grazed during the past year to ensure that the presence of other species of trichostrongyles would not complicate interpretation of the response to the *H. contortus* vaccine. Preliminary investigations with

tracer lambs during October 2003 confirmed that only very low levels of trichostrongyles were present. During December, each paddock was contaminated by grazing with 15 yearling wethers, each of which had been infected with 5000 "Kirby" *H. contortus* larvae. "Kirby" *H. contortus* is an anthelmintic susceptible isolate recovered from the Armidale locality (Albers and Burgess, 1988), and since it has been maintained by passage through sheep at McMaster Laboratory. At the end of December the wethers were drenched and returned to the station flock.

On 10 December 60 further wethers were drawn from the 2003 station Merino lamb crop and weaned at 3–4 months of age. Thirty were vaccinated with *H. contortus* antigen and 30 were injected with adjuvant only. The lambs were then moved to a separate pasture which had been spelled to ensure low levels of larval contamination and kept there for a month while pasture burdens of infective *H. contortus* larvae increased on the seeded experimental paddocks. The lambs were re-vaccinated on January 13, and then the plan was to move 15 vaccinates or 15 controls to each freshly contaminated experiment paddock, where they would remain for the duration of the trial. The experimental design is set out in Table 1.

It was considered at the outset that any farmer in the future using vaccination to control *Haemonchosis* would be advised to immunise the whole of his flock in order to gain maximum epidemiological benefit. The present trial was set up in an attempt to measure this potential effect and so the groups of immunised and control sheep grazed separate rather than shared paddocks.

While on the experiment paddocks the sheep were inspected daily for clinical signs of haemonchosis. Twice a week the animals were gently driven over a distance of approximately 100 m to identify any weak individuals; those so identified were treated with anthelmintic. Faeces for egg counts and blood for packed cell volumes and serology were collected from all sheep at approximately 3 week intervals.

Every 2 weeks between January and April 2004 a pair of worm free tracer lambs was introduced to each paddock, removed 14 days later then housed for a further fortnight before being killed for worm counts. Daily larval infection rates were calculated by dividing the total number of worms recovered by 14.

The following criteria were decided at the outset of the trial:

- (1) any animal with a PCV equal to or less than 15% would be treated with a short acting anthelmintic

Table 1
Design of experiment

Date	Activity
1 December 2003	Fifteen infected wethers introduced to each of four experimental paddocks to seed them with <i>Haemonchus</i> larvae
10 December	Sixty, 3–4-month-old lambs, weaned. Thirty vaccinated with antigen, 30 given adjuvant only. All moved to separate spelled pasture
29 December	Infected wethers removed from experimental paddocks
7 January 2004	Diarrhoea outbreak in lambs, some deaths
13 January	Lambs re-vaccinated as on 10 December. Moved in equal numbers to two vaccinate only or two control only paddocks, but further deaths unbalanced these numbers (see this table)
3 February to 11 May	Regular monitoring of WECs and PCVs. Re-vaccination if WECs of vaccinates began to rise. Drenching if PCVs below pre-set criteria. Monthly estimation of pasture contamination using tracer lambs

(levamisole or ivermectin), left to graze with its paddock mates and that any subsequent data from that individual would be discarded from the analysis;

- (2) once six individuals in either the vaccinated or control groups had required anthelmintic, then all of that group would be treated and the subsequent data obtained from them would be included in the analysis;
- (3) further boosts would be administered to the vaccine group if the egg count and/or PCV data suggested that vaccine immunity was waning.

3. Results

3.1. General

An outbreak of diarrhoea followed by ill thrift developed in the lambs. Some were affected before they were introduced to the experimental paddocks, others shortly thereafter. Ten vaccinated and six control lambs died as a result ($p > 0.05$), but the cause of death was not

known, though WECs revealed that it was not nematodiasis. Moderate numbers of coccidia were found in the faeces and treatment with *Tribactral Injection*® (2 ml/day, Jurox Pty. Ltd.) resulted in an improvement in the animals' condition. The outcome was that before the sampling was made, the number of surviving lambs was 8 and 12 in the vaccine paddocks and 11 and 13 in the control paddocks. As a consequence of these deaths and the individual anthelmintic treatments described below, the number of animals in each group from which data was available for analysis at each sampling time is presented in Table 2.

3.2. Pasture infectivity

The rainfall recorded during the trial approximated to the seasonal average (Fig. 1) and from December there was clearly adequate moisture for larval development (until about mid November overnight temperatures in the Armidale locality are usually below 10 °C and too low for *H. contortus* larvae to develop) (Gibson and Everett, 1976).

Table 2
Number of vaccinated and control lambs at each sampling point

Group	Category	Day relative to first vaccination							
		0	55	80	90	105	127	142	154
Vaccinates	Total <i>N</i>	30	20	20	20	20	20	20	20
	<i>N</i> died since preceding sample	0	10 ^a	0	0	0	0	0	0
	<i>N</i> treated since preceding sample	0	0	0	0	0	0	2	0
	<i>N</i> available for analyses ^b	30	20	20	20	20	20	18	18
Controls	Total <i>N</i>	30	24	24	21	20	18	17	16
	<i>N</i> died since preceding sample	0	6 ^a	0	3	1	2	1	1
	<i>N</i> treated since preceding sample	0	0	0	4	2	20	0	6
	<i>N</i> available for analyses ^b	30	24	24	18	16	16	15	10

^a Died of causes other than *Haemonchosis*.

^b Data from lambs requiring individual anthelmintic treatment were excluded from subsequent analysis, except in the control group after they were all treated on day 105.

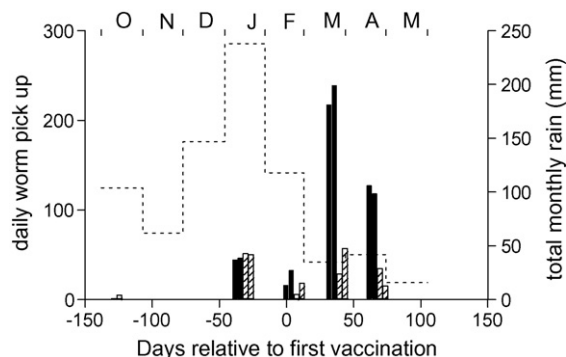


Fig. 1. Rainfall and paddock infectivity. Bars show the mean number of *H. contortus* acquired per day by the tracers on individual paddocks. Solid bars: control paddocks; hatched bars: vaccinates' paddocks; dotted line: total monthly rainfall.

The time course of the estimated daily infection rate of *H. contortus* on each paddock is plotted in Fig. 1. During October fewer than five *Haemonchus* per day were acquired by each tracer lamb on any paddock, but, after being seeded by the yearlings in December, this rose to between 40 and 50 worms per day in January, with each paddock having very similar levels of contamination. During February the pick up rate seemed to fall, but there was no obvious difference between the control and vaccine paddocks. In contrast, the infectivity of both control paddocks was much higher than the vaccine paddocks during March ($p < 0.001$) and April ($p < 0.001$). Whereas the vaccine paddock pick-up rate was maintained within the 15–55 larvae per day range, values in the control paddocks rose to more than 200 and 100 per day during March and April, respectively.

Small numbers of *Teladorsagia* spp. were also acquired throughout the trial (range 1–13 per day), but no significant differences in numbers of this parasite were observed between the tracers grazing vaccine or control paddocks (not shown) at any time point.

3.3. Faecal egg counts

Low numbers of WECs were detected in the faeces of most control sheep 21 days after turnout (Fig. 2a). Thereafter the counts rapidly increased, climbing to a peak overall mean of 30,537 epg faeces by day 105 after the first vaccination, that is 71 days after entry to the parasite contaminated paddocks. In accordance with the pre-set plan and because six controls had been drenched by this time, all the control group were treated with 200 $\mu\text{g/kg}$ ivermectin on day 105. Mean control egg counts subsequently plummeted to a low of 150 epg by day 127, but rapidly picked up again reaching mean

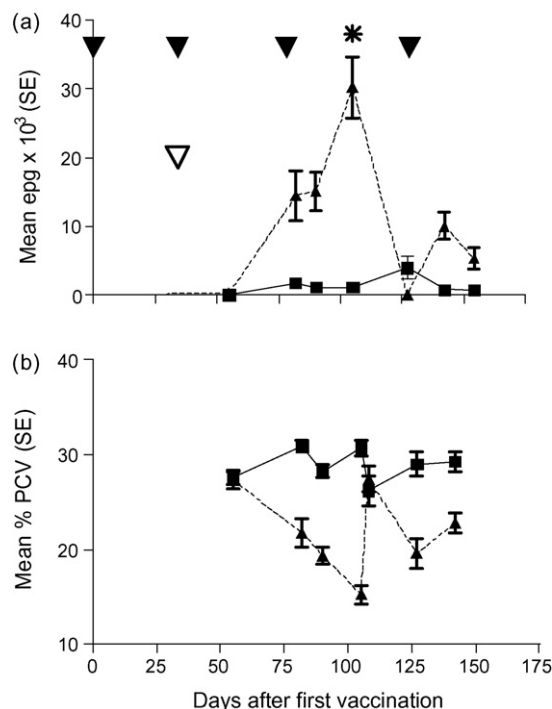


Fig. 2. Worm-egg counts and anemia. (a) Worm-egg counts (eggs g^{-1}); (b) packed cell volumes (%). Squares joined by solid lines: mean and standard error for all vaccinated sheep; triangles joined by broken lines: mean and standard error for all control sheep; large filled triangles: vaccination days; open triangle: day sheep turned onto the paddocks; asterisk: day when the entire control group were treated with anthelmintic.

values of 10,173 and 5620 epg on days 142 and 154, respectively.

Mean egg counts for the vaccinated lambs are also shown in Fig. 2a. Most vaccinates had positive egg counts by day 80 of the trial and in three lambs these exceeded 5000 epg and so it was decided to administer another vaccine boost. The overall mean egg count in the vaccinates then remained at about 1200 epg until day 127 when it rose to 3850 epg, a level which prompted the third and final vaccine boost. From then until the end of the trial mean counts in the vaccinates stayed below 800 epg.

Statistical analysis revealed highly significant effects due to vaccination ($p < 0.001$), but there was no evidence that paddock contributed any variability to the data ($p > 0.05$).

3.4. Signs of haemonchosis

3.4.1. Deaths

Despite careful monitoring, 8 of 24 lambs in the control groups died with high faecal egg counts and/or

low PCVs. Four died before and four after the whole control group had been treated (Table 2).

No vaccinated lambs died of haemonchosis, an outcome which significantly differed from the control group ($p < 0.01$).

3.4.2. Individual salvage anthelmintic treatments

Twelve individual anthelmintic treatments were given to sheep in the control group during the course of the trial (two lambs had to be treated twice). Of these, half of the treatments were administered before the group therapy given on day 105, the rest on day 142 (Table 2). Three treated lambs subsequently died. Significantly fewer individual treatments were given to the vaccine group ($p < 0.01$), since only 2 of the 20 vaccinated animals were treated, both on day 127.

3.4.3. PCVs

Most lambs in both groups were mildly anaemic when first tested 3 weeks after entry to infected paddocks (day 55) when the overall control and vaccinated group mean PCVs were 27 and 28%, respectively (Fig. 2b).

The control sheep then became progressively more anaemic, dropping to an overall mean PCV low of 15% by day 105, when the whole group was treated with ivermectin. Control PCVs then recovered, climbing to a mean close to the starting value on day 127, but soon fell again to values of 20 and 23% on days 142 and 154, respectively.

The mean PCV of the vaccinates remained close to its starting value throughout the trial. Mean control PCVs were always significantly lower ($p < 0.05$) than vaccinate values, except at the initial sampling day and on day 127, the first bleed after the whole control group had been treated. Aggregating these effects over the life of the trial, the difference between the two groups was highly significant ($p < 0.001$), but there was no evidence that paddock contributed any variability to the PCV data.

3.5. Serology

Each serum sample was tested in two ELISAs using plates coated with VA (the antigen preparation used to vaccinate the sheep) and *H. contortus* SP3. Total antibody as well as IgG1, IgG2, IgM, IgA and IgE class specific antibody titres were determined for both antigens.

3.5.1. Vaccine antigen responses (Fig. 3)

The vaccinated lambs responded vigorously to the first two immunizations such that by day 55 (21 days after the first boost) mean total immunoglobulin titres

approached 9000 (Fig. 3a). However by day 75 this value had declined to about 4000. Subsequent immunizations on days 80 and 127 rapidly stimulated further peaks which each exceeded 15,000, but on each occasion these subsequently waned to about 5000 within 2 or 3 weeks. Total antibody titres detected in the control sheep were markedly lower than in the vaccinated sheep throughout the trial, although they did increase significantly as the experiment progressed.

The profiles of the IgG1, IgG2 or IgM responses in the vaccinated sheep broadly mimicked those observed with total antibody (Fig. 3b–d). Much lower, highly variable titres of IgA and IgE antibody were also detected in the vaccinated sheep, especially after the third and fourth immunizations (Fig. 3e and f). For each isotype the titres recorded in the control lambs were always much lower than in the vaccinated group. In these animals, low but distinct responses were detected for all isotypes except IgM.

3.5.2. Correlation between vaccine responses and faecal egg counts

The third and fourth vaccine doses administered on days 80 and 127 stimulated rapid rises in antibody titre which were followed by rapid falls in egg output. Similarly, when antibody titres waned a few weeks after the second and third vaccine doses, increased egg counts were observed (the fourth vaccine dose was administered too close the end of the trial to determine whether the same relationship existed then as well) (Fig. 4). Regression analysis revealed significant inverse correlations between total anti-vaccine titres and egg counts immediately prior to V3 ($p < 0.05$) or V4 ($p < 0.01$). When the same analysis was conducted with IgG subclasses, a significant correlation was obtained with IgG1, but not with IgG2 (Fig. 5). Similarly, no significant correlations were observed between either IgM, IgA or IgE titres and egg counts on either of these occasions.

3.5.3. Larval antigen (SP3) responses

Mean total antibody peaks exceeding 20,000 were observed in the vaccinated lambs following each of the three booster injections (Fig. 6). In contrast the mean peak titre attained by the control sheep was less than 1600.

The IgG subclass response followed the same general pattern although IgG1 peaked between V2 and V3 whereas IgG2 peaked after V4. From about mid way through the trial much lower total, IgG2 and IgG1 responses were detected in the unvaccinated lambs.

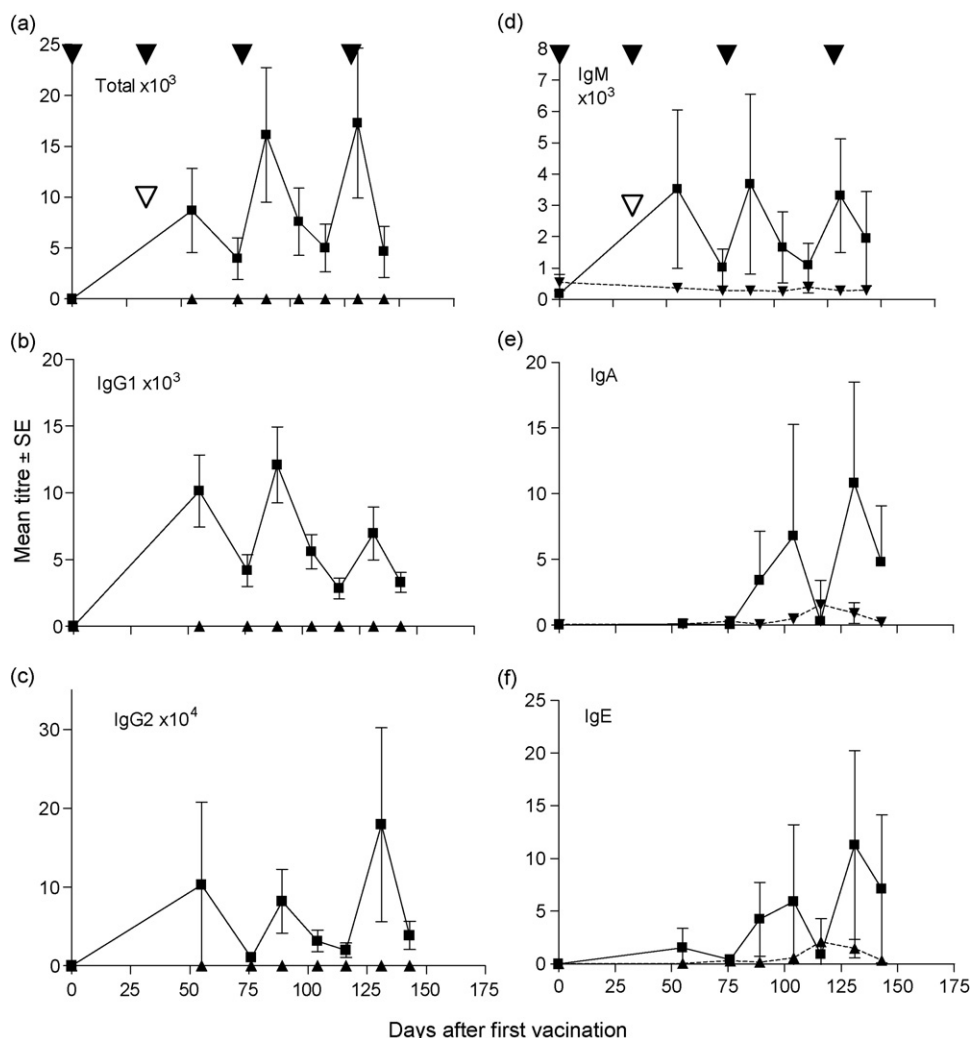


Fig. 3. Anti-vaccine antigen responses. Each panel shows the mean and standard error total or isotype antibody responses of the vaccinated and control sheep to the vaccine antigen. Solid filled triangles: vaccination days; open triangles: day sheep turned onto the paddocks; squares or triangles joined by solid lines: vaccinated sheep; triangles joined by broken lines: control sheep.

The IgM anti-larval response of the vaccinated sheep was broadly similar to the IgG1 profile, tailing off towards the end of the experiment. Pre-trial samples gave relatively high background values.

During the latter half of the trial much lower IgA and IgE responses were detected in both vaccinated and control sheep. In contrast to the other isotypes, mean control values nearly always exceeded those of the vaccinated sheep (Fig. 6).

4. Discussion

The trial clearly showed that vaccination with the native *H. contortus* intestinal glycoproteins, H-gal-GP and H11, in combination with QuilA adjuvant conferred

substantial benefits to Merino weaner lambs grazing pasture infected with *H. contortus*. Vaccination substantially reduced haemonchosis: more specifically, the number of deaths, the number of salvage anthelmintic treatments and the degree of anaemia were all significantly reduced in the immunised group compared to the controls. As a consequence of vaccination reducing worm-egg counts, pasture contamination was greatly diminished in the paddocks grazed by the vaccinated lambs during the latter part of the trial.

The fact that one third of the control lambs died of haemonchosis during the trial despite careful monitoring suggests that the criteria chosen before drug treatment was given should have been more stringent. A new condition instituted for *H. contortus* infected

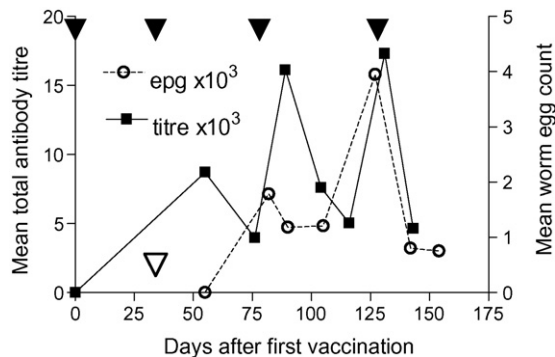


Fig. 4. Vaccinates: egg counts and antibody titres. Solid filled triangles: vaccination days; open triangles: day sheep turned onto the paddocks; squares joined by solid lines: total antibody titre of vaccinated sheep; circles joined by broken lines: worm-egg count of vaccinated sheep.

animals by our laboratory animal ethics committee is that every day they must be driven gently 100 m to identify weak animals.

The protective immunity stimulated by each vaccine boost was relatively short lived, lasting about 7 weeks. Indeed, it was the rising egg counts in some vaccinated sheep which prompted the vaccine boosts given on days 80 and 127, 46 and 49 days after the second and third vaccine doses, respectively. Retrospective serology

showed that the anti-vaccine antigen titres were quite variable between individual lambs, possibly due to genetic variation or other unknown factors. Interestingly, worm-egg counts were inversely correlated with anti-vaccine antigen titres prior to the vaccine boosts and that the effect was due to the IgG1 rather than the IgG2 response. Published sheep trials using either H11 or H-gal-GP as individual vaccine antigens have also shown that protection was correlated with serum antibody (Smith et al., 1999) and that it could be passively transferred either by immune serum (Smith, 1993) or colostrum (Andrews et al., 1995). This last result provided indirect evidence that IgG1 alone could mediate vaccine protection, since this isotype substantially predominates in ovine colostrum (Pfeffer et al., 2005). The role of the IgG2 anti-H11 or anti-H-gal-GP antibodies in the protection observed here is uncertain, but it may be relevant that, compared with IgG1, a greater proportion of the IgG2 anti-H-gal-GP moiety was directed against glycan epitopes (Geldhof et al., 2005).

Comparison of the kinetics of the anti-vaccine antibody and egg count profiles in Fig. 4 suggested that a rise in antibody concentration was followed by a rapid fall in egg count whereas a subsequent decrease was followed by a more gradual increase in egg output. Previously published data suggests that very high

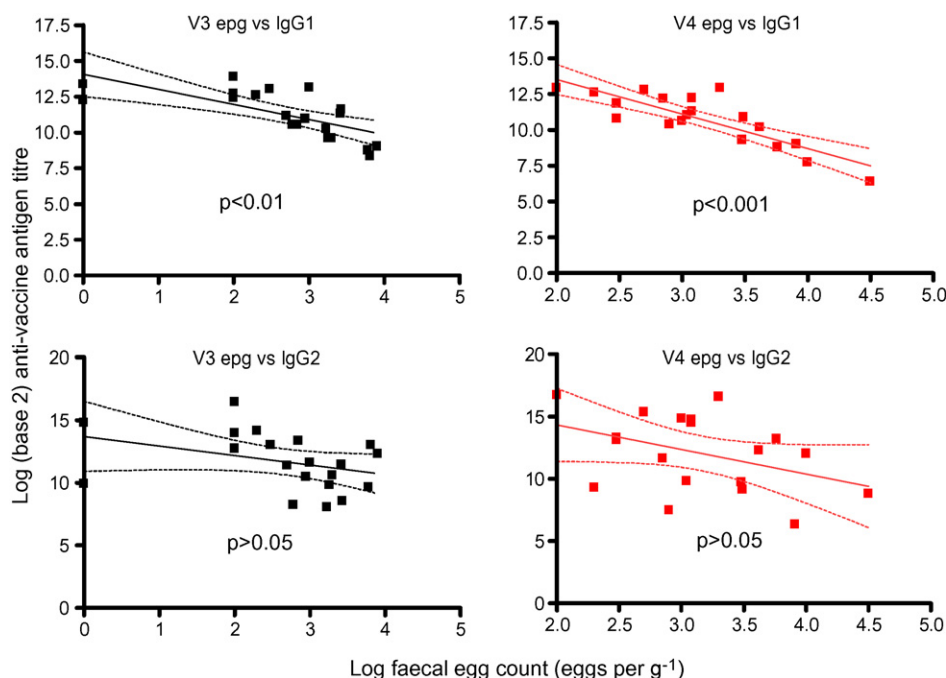


Fig. 5. IgG1 or IgG2 anti-vaccine antigen titres vs. egg counts prior to V3 and V4. IgG1 or IgG2 anti-vaccine antigen antibody titres of individual immunised sheep plotted against the faecal egg count on the last sampling date prior to the third or fourth vaccination. Lines of best fit (solid) are plotted with their 95% confidence intervals (broken).

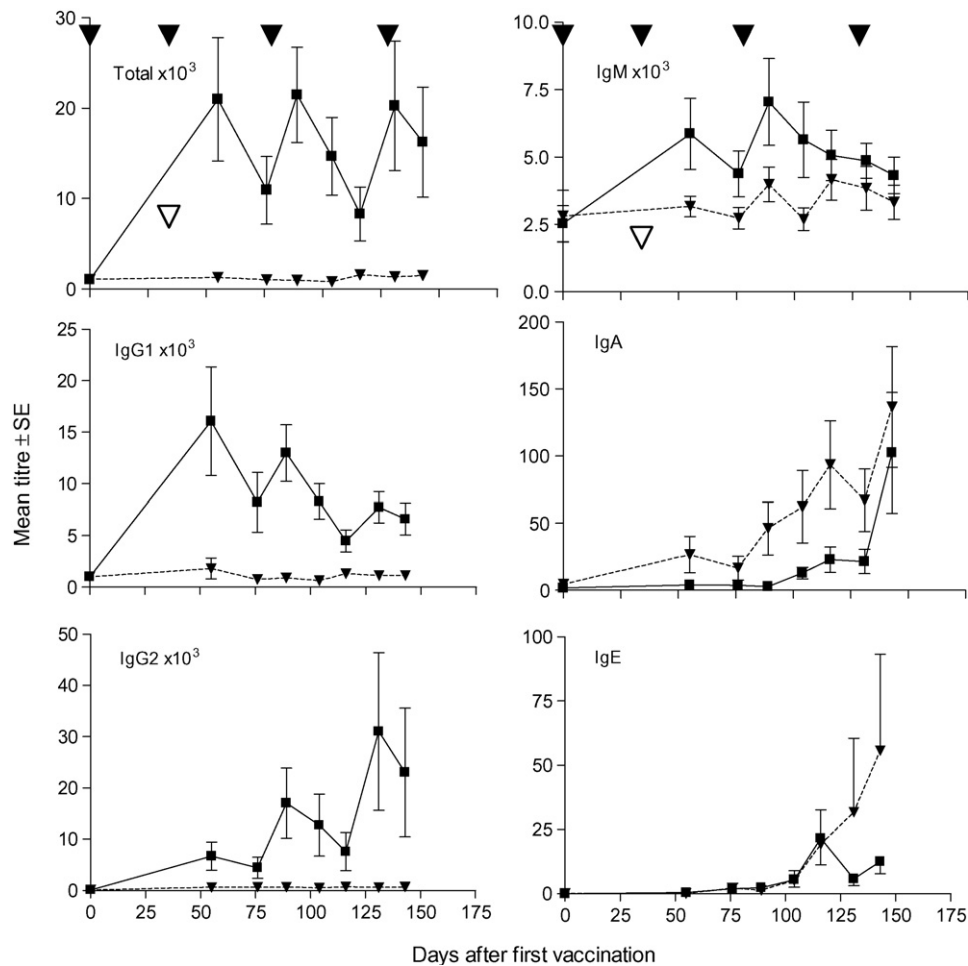


Fig. 6. Anti-SP3 antigen antibody responses. Each panel shows the mean and standard error total or isotype antibody response of the vaccinated and control sheep to the larval antigen. Solid filled triangles: vaccination days; open triangles: day sheep turned onto the paddocks; squares joined by solid lines: vaccinated sheep; triangles joined by broken lines: control sheep.

antibody titres would lead to a rapid loss of fecundity as well as expulsion of late L4 and adult parasites (Smith and Smith, 1993). However, after a decrease in antibody titre, there would be delay roughly equivalent to the prepatent period before enough incoming L3s or early L4s matured into sufficient adult worms for the egg count to rise.

The question as to whether *H. contortus* gut membrane glycoproteins like H11 and H-gal-GP are “hidden”, i.e. not recognised by the host during infection, is the subject of some debate (reviewed by Smith and Zarlenga, 2006). The “hidden” versus “natural” antigen concept is not just academic because, if natural boosting occurred, the duration of vaccine immunity could be greatly increased. *H. contortus* gut membrane proteins were originally described as “hidden” because immune sheep which had been

exposed to prolonged infections did not recognize them serologically (Smith, 1993), but contradicting data has since been published (Andrews et al., 1995; Jasmer et al., 2003). In the present trial continuous exposure of the vaccinated sheep to incoming *H. contortus* did not maintain the high concentrations of vaccine antigen antibody needed to maintain protective immunity, indicating that from a practical perspective H11 and H-gal-GP did indeed behave as “hidden” antigens. This agrees with the results of an earlier vaccine trial with grazing sheep (Smith et al., 2001a,b). However, low-level anti-H11/H-gal-GP antibody titres were detected in the control lambs from 12 weeks after exposure suggesting either that these antigens are not completely hidden, that H11/H-gal-GP possess epitopes which are shared by natural antigens, or that “hidden” vaccine antigens may have become available as a result of

decomposition of dead worms. The observation that the vaccinated sheep possessed much higher IgG anti-SP3 antigen titres than the controls (Fig. 6) increases the plausibility of the cross-reaction theory.

The SP3 antigen, as a crude homogenate of *H. contortus* L3, would consist of a mixture of soluble proteins. A similar preparation from *Trichostrongylus colubriformis* L3 has been shown to contain a large number of immunogenic epitopes as identified by plasma from resistant lambs (Kiel et al., 2007). In the present trial it was clear that immunization with the VA stimulated an antibody response which reacted with SP3. The likelihood of the VA actually being present in SP3 is remote because it has been shown that transcripts encoding the amino-, metallo- or aspartyl-protease components of H11 and H-gal-GP were confined to fourth and adult stage *H. contortus* and absent from eggs and L3 (Longbottom et al., 1997; Redmond et al., 1997). Furthermore, hyperimmunization of sheep with extracts of *H. contortus* L3 similar to SP3 did not confer any protective immunity (Smith, 1977). The obvious explanation is that there are cross-reacting epitopes present in both antigen preparations.

Whereas IgG1 responses to SP3 in vaccinated lambs were marginally higher than those for VA, IgG2 responses to SP3 were markedly lower. These differences may further reflect the relative importance of IgG1 and IgG2 for protection against *H. contortus*. Although in this study it was not possible to make direct comparisons between the IgG1 and IgG2 responses because of potential differences in binding characteristics of the individual monoclonal antibodies, there are other reports that suggest relatively more systemic IgG1 rather than IgG2 is generated during infection in resistant animals. Gill et al. (1993) found that the major antibody isotype in the plasma of genetically resistant sheep during artificial challenge was IgG1, and that the lower levels of IgG2 did not appear to be associated with enhanced resistance to *H. contortus*. Similarly, Gomez-Munoz et al. (1999) have shown that sheep infected with *H. contortus* generated a systemic antibody response that was principally IgG1.

In contrast to IgG1 and IgG2, IgA and IgE responses appeared to be more associated with the natural infection from pasture than vaccination. VA specific IgA responses remained at low levels (titres less than 20) for the entire experiment whereas those for SP3 increased over time and were higher in control lambs. This could reflect the greater larval contamination of the plots grazed by the unvaccinated lambs and a response to the natural antigens generated by the continuous infection rather than the vaccine. The highest levels of

IgA occurred after the anthelmintic treatment and at a time when WEC data indicated that some degree of resistance had developed in control animals. IgE responses in general followed a similar pattern to those of IgA, but occurred later and were of a lower magnitude. Increased levels of IgA and IgE have been associated with reduced WEC and worm burdens to gastrointestinal nematodes (Gill et al., 1993; Bendixsen et al., 2004) and reduced fecundity of *H. contortus* (Strain and Stear, 2001). However, the systemic occurrence of these locally produced antibodies, as found for IgA (Sinski et al., 1995), may not necessarily be highly correlated with their levels in tissue. This may be related to the time at which samples are taken. Windon (unpublished) found that in animals given a series of bolus challenges with *T. colubriformis*, there were spikes of IgA response 10–14 days after a primary challenge which decreased in magnitude with subsequent challenges. In contrast, there was a small IgE peak 14–16 days after a primary challenge, which increased in magnitude and occurred earlier with subsequent challenges so that in highly immune animals there was a large IgE peak within 7 days of challenge.

In the present trial, the level of protection conferred by the experimental vaccine against *H. contortus* easily exceeded that predicted to offer a useful level of control (Barnes et al., 1995). It was therefore concluded that if a similar result could be obtained with a recombinant version of some of the proteins in the vaccine cocktail then the prospects for a commercial vaccine were real. Efforts to produce protective recombinants derived from H11 and H-gal-GP have so far proved elusive, but are still being actively pursued. Furthermore, the vaccine's commercial attractiveness would be enhanced if used with an adjuvant that provided more prolonged activity with a stronger IgG1 response than QuilA.

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