final report

Project code: B.AHE.0214

Prepared by: W.D. Smith

Moredun Research Institute

Date published: January 2014

ISBN: 9781925045826

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

A commercial vaccine for Barber's pole worm – further development

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Five field trials were conducted in NSW with "Barbervax", which is a novel vaccine for controlling Barber's Pole worm, an important parasite of Australian sheep. These trials were an essential part of the work needed to obtain commercial registration of the vaccine. Two trials with lambs confirmed the efficacy of the vaccine and demonstrating that it also conferred an epidemiological benefit. Three trials with hoggets showed similar efficacy, extending the potential use of the vaccine to that class of sheep. All the data will be added to an existing dossier, currently under review by the APVMA.

Once approved, Barbervax should offer the Australian sheep industry an additional, sustainable tool to control one of its most important parasites.

Executive summary

The Barber's Pole worm, *Haemonchus contortus*, is an important gastrointestinal parasite of sheep and goats in Australia and overseas. Because the parasite prefers warm moist conditions, Haemonchosis is particularly common in the summer rainfall zone especially in North Eastern NSW and Southern Qld, but the disease can occur sporadically in any State. *Haemonchus* is usually controlled by anthelmintic drugs, but strains resistant to these chemicals are common and widespread in endemic areas. Alternative methods for control are required. Vaccination could theoretically offer a solution, but no vaccines are currently registered for any gut dwelling worm parasite of any host.

The project to be described is the second part of a three stage objective where the overall aim is to make an effective Barber's Pole worm vaccine commercially available for Australian sheep producers. This vaccine was developed at the Moredun Research Institute in Scotland following some 20 years work and will be manufactured by one of its subsidiaries, Wormvax Australia, at the Department of Food and Agriculture, Western Australia laboratory in Albany WA .

The first part of the overall project was mainly concerned with obtaining a Good Manufacturing Practice licence for the vaccine and with determining vaccine efficacy for lambs in field trials. Most of the trials were done in New England, where *Haemonchus* is endemic and a serious problem. The results were successful and culminated in the submission of a dossier to the Australian Pesticide and Veterinary Medicines Authority (APVMA) seeking permission to sell the vaccine as an aid to controlling Barber's Pole in lambs. This first part of the project will be complete when approval of the dossier has been granted. A decision is not expected until August 2014 after which this part of the project will be reported in full.

The present project forms the second part of the overall objective and contains results which show that the vaccine affords an epidemiological benefit by reducing pasture contamination with infective worm larvae. In addition, it shows that the vaccine is effective in yearling sheep. As soon as possible after the current dossier has been approved, this information will be added to it so that its overall scope is broadened and strengthened.

The third part of the project is currently still in the animal trial phase. Its main aim is to determine the ability of the vaccine to confer protection on ewes, both in terms of suppressing their increased susceptibility to infection around lambing and during lactation, an important source of infection for their lambs, but also during the high risk period in late summer when deaths due to Barber's Pole worm are not uncommon in ewes. If these results are favourable, the data will also be added to the dossier.
Therefore if all the trials are successful and approval of the extended dossier is obtained, farmers will be able to use the vaccine in lambs, yearlings and ewes. By these means it is hoped that Australian farmers will have a new tool to combat Barber's Pole worm across their entire flock.

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1 Background

Commercialising a vaccine to control Barber's Pole worm in Australia and beyond.
The Barber's Pole worm, *Haemonchus contortus*, is an important gastrointestinal parasite of sheep and goats both in Australia and overseas. Because the parasite prefers warm moist conditions, Haemonchosis is particularly common in the summer rainfall zone especially in North Eastern NSW and Southern Qld, but fatal disease can occur in any State. *Haemonchus* is usually controlled by anthelmintic drugs, but strains resistant to these chemicals are common and widespread in endemic areas. Alternative methods for control are required. Vaccination could theoretically offer a solution, but no commercial vaccines are currently registered in any country for any gut dwelling worm parasite of any host.

After some 20 years of work, a promising *Haemonchus* vaccine called "Barbervax" has been developed at the Moredun Research Institute in Scotland. Important questions to answer were whether Barbervax would work under Australian conditions and, if so, whether it could be successfully commercialised for use in Australia and other countries.

2 Project objectives

A three phase approach

Overall objective

This report describes the second phase of a three part objective where the ultimate aim is to make an effective Barber's Pole worm vaccine commercially available for Australian sheep producers. In order to provide context, the objectives of Phases 1 and 3 of the project are also outlined below, but these phases will be reported separately.

2.1 Phase 1 (MLA project B.AHE.0068)

The first part of the overall project was mainly concerned with obtaining a Good Manufacturing Practice licence for the vaccine and with determining vaccine efficacy for lambs in field trials. Most of the trials were done in New England, where *Haemonchus* is endemic and a serious problem. The results were successful and culminated in the submission of a dossier to the Australian Pesticide and Veterinary Medicines Authority (APVMA) seeking permission to sell the vaccine as "an aid to controlling Barber's Pole in lambs". This first part of the project will be complete when approval of the dossier has been granted. A decision is not expected until August 2014 after which this part of the project will be reported in full.

2.2 Phase 2

The present, second, part of the overall objective, was done to answer two main questions. First, would the vaccine afford an epidemiological benefit by reducing pasture contamination with infective worm larvae and, second, would it be effective in yearling sheep? It was necessary to answer the second question because the APVMA requires that a vaccine is shown to be effective for all classes of sheep before it can be registered for use across a whole flock.

2.3 Phase 3 (MLA project B.AHE.0232)

The aim of the third part of the project is to determine the ability of the vaccine to confer protection on ewes, both in terms of suppressing their increased susceptibility to infection around lambing and during lactation, an important source of infection for their lambs, but also during the high risk period in late summer when deaths due to Barber's Pole worm are not uncommon in ewes.

3 Methodology

Design of replicated plot trials in which vaccinated and control lambs grazed separately

Please refer to Appendices 1 and 2 for full details of the design of these two trials

Design of trials with yearling sheep

Please refer to Appendices 3, 4 and 5 for full details of the design of these three trials

4 Results and Discussion

4.1 Replicated Plot Trials with lambs

The aim of these trials was three-fold:

- to confirm the protective results with lambs obtained with Barbervax during the 2011-2012 summer (project B.AHE.0068),
- to determine whether the protective effect would be improved if vaccinated and control lambs grazed separately (farmers would be advised never to graze unvaccinated animals with vaccinated ones) and
- to show the downstream epidemiological benefit of vaccination by demonstrating reduced pasture contamination.

Interestingly, opinion on the need for the third objective was divided amongst experienced Australian parasitologists: some thought that if Barbervax substantially suppressed egg output, it was obvious that reduced pasture contamination would follow and that a formal demonstration was unnecessary. Others, however, felt that some APVMA reviewers would demand concrete evidence of reduced contamination and so it was considered paramount to cater for that view.

Both trials followed a very similar design with vaccinated and control lambs weaned and placed on separate but adjacent plots. To counter the argument that any differences between plots might be due to variation in initial larval contamination rather than the effect of the vaccine, each trial was replicated four times. The plan was that four plots would contain ten vaccinated sheep each and ten controls would graze in each of four further plots.

For obvious reasons this design would have been difficult and expensive to run on a private farm, but fortunately Veterinary Health Research and CSIRO, Chiswick had existing sets of replicated plots on their premises, located about 20km North and South of Armidale, respectively. Therefore these organisations were subcontracted to run these trials

Although it was a dry spring in the Armidale vicinity, rain from late December ensured a good natural challenge of Barber's Pole worm at both sites.

Overall the results were clear cut in both trials – **see Appendices 1 and 2 for detailed reports of each tria**l.

Compared to the control lambs, *Haemonchus* egg counts were greatly reduced in the vaccinates, their degree of anaemia and need for precautionary drenching was much less and larval contamination on the vaccinate plots was significantly suppressed.

There were some differences between the trials, however. *Haemonchus* egg counts peaked some 4 weeks earlier at VHR compared to CSIRO, where the peak counts were substantially higher. Whether this reflected genetic differences in the susceptibility of the sheep, local climatic variation, sward quality (when both sites were visited in March 2013 the VHR pasture was much the lusher) or other unknown factors is by no means clear.

The overall reduction in the *Haemonchus* egg output of the vaccinates was higher at CSIRO (91%) than at VHR (71%), despite antibody titres being in the same order at both premises. This CSIRO result did suggest that grazing vaccinates alone might be beneficial because the trial conducted at the same premises with the same sheep as lambs all grazing the same paddock during the previous summer recorded an overall protection figure of (84.4% - Table 1). This difference is in the same order as that predicted by mathematical modelling which revealed that grazing vaccinates and controls apart offers a 10% increased benefit to the vaccinates (R. Dobson, personal communication). However, the VHR data did not demonstrate the same effect because at 71% the level of protection was lower than that recorded in all three 2011-12 trials where vaccinated and control lambs shared the same pasture.

It was concluded that the first and third aims of these trials were achieved successfully, but the second aim, namely to determine whether the protective effect would be improved if vaccinated and control lambs grazed separately, had not been attained with certainty.

4.2 Trials with yearlings

The main purpose of these trials was to provide data for registration purposes to show that Barbervax could be useful for controlling *Haemonchus* in adult sheep and that four immunisations 6 weeks apart were adequate for achieving this over the high risk summer season. The trials were done on three separate premises, one of which was the CSIRO property where one of the above replicate plot trials ran simultaneously. See Appendices 3, 4 and 5 for detailed reports of each trial.
The vaccinated sheep in all the yearling trials had received a course of Barbervax the previous summer when they were lambs, the last dose of vaccine being administered some 8 to 9 months previously. Previous results had indicated that sheep which had received Barbervax as lambs retained immunological memory for at least 10 months (Field trial 1 in the dossier submitted to the APVMA April 2013) and so four

immunisations each given 6 weeks apart were predicted to be sufficient for season long protection.

This prediction seemed to be correct in that Haemonchus egg counts were significantly depressed in vaccinated sheep compared to controls at all 3 sites (Table 1, but see individual reports for more details). Interestingly, the percent protection (eggs) ranged from 64.8 to 84.4%, which was within the same range found in the same sheep when they were lambs a year earlier, but there was no evidence that protection was consistently better on any one property (Table1).

Control egg counts averaged over the season were lower when the sheep were yearlings rather than lambs, presumably because a degree of natural immunity had been acquired in the first grazing season. That this difference was simply due to 2012-13 being the wetter, giving more favourable conditions for the parasites, is not supported by the data from the control lambs grazing a mere 300 metres away in the CSIRO replicated plot trial (Table1).

Table 1. Comparison of average control egg counts and percent protection of yearlings and lambs at 3 trial sites over consecutive summers

		%Protection (eggs)		
Summer	**Sheep**	**CSIRO**	**Kingstown**	**Dundee**
2011-12	Lamb	84.4	83.6	64.8
2012-13	Yearling	63.7	70	82.2
2012-13	lamb	91.0		

		Mean control epg		
Summer	**Sheep**	**CSIRO**	**Kingstown**	**Dundee**
2011-12	Lamb	3267	2894	2451
2012-13	Yearling	1974	1723	745
2012-13	lamb	4013		

Compared to the trials conducted during the previous summer when the sheep were lambs, anaemia was less marked in the controls and relatively few required a precautionary drench. Presumably this reflected the fact that they had fewer worms than when lambs (see mean control egg counts in Table 1), but also because, being heavier, they were more resilient to blood loss caused by the worms.

It was interesting that antibody titres were higher in the vaccinates than the controls at the start of every trial, despite the fact that 8-9 months had elapsed since their last vaccine boost the previous autumn. The half-life of circulating IgG in the sheep is only 14 days, indicating that these elevated titres were likely to have been the result of further antigenic stimulation rather than being residual from the previous course of vaccine. It was concluded that the vaccine antigens must not be entirely "hidden"; possibly some of vaccine antigen epitopes are shared with those on the natural antigens which the sheep respond to when they ingest gastro-intestinal nematodes.

Whatever their cause, these titres were not associated with any protective immunity at the start of any of the trials because the egg counts of both groups were very low at that stage.

It was concluded that the main objective had been met since all three trials showed that Barbervax could provide yearling sheep with a useful degree of protection against Barber's Pole worm and that this could be achieved with four doses of vaccine instead of five.

5 Success in achieving objectives

5.1 Trials with lambs

It was concluded that:

1) the protective results with lambs obtained with Barbervax during the 2011-2012 summer were confirmed

2) that vaccination caused a downstream epidemiological benefit by demonstrating reduced pasture contamination

Therefore two of the three objectives were achieved successfully, but the third, to determine whether the protective effect would be improved if vaccinated and control lambs grazed separately, had not been attained with certainty.

5.2 Trials with yearlings

It was concluded that Barbervax could provide yearling sheep with a useful degree of protection against Barber's Pole worm and that this could be achieved with four doses of vaccine instead of five if the sheep had received a course of the vaccine when lambs.

Thus both objectives had been achieved successfully.

6 Impact on Meat and Livestock Industry – now and in five years time

If approved by the APVMA, Barbervax will provide Australian graziers with a completely new way of controlling Barber's Pole worm in their lambs.

The plan is for a limited launch of the vaccine in time for the 2014- 2015 summer worm season. Initially it will only be licensed for use in lambs, but hopefully the data in the present report will allow this to be extended to yearlings and, if the parallel phase 3 trials (project B.AHE.0232) currently underway are successful, permission should be granted for use in ewes, perhaps by the following year.

A major advantage of Barbervax is that, unlike most anthelmintics, it is sustainable. Vaccine resistant worms are most unlikely to evolve. This should also help preserve the life of those anthelmintics which are still effective.

It is hoped that in 5 years time Barbervax will be an important component of integrated worm management strategies in *Haemonchus* endemic areas.

We would also expect Barbervax to be exported to certain other countries e.g. South Africa, Brazil and Uruguay within the next 5 years.

Probably the weakest aspect of Barbervax is the fact that it has to be given 5 times over the season to protect lambs and 4 times to protect yearlings. Efforts to increase the duration of immunity using different adjuvants and or slow release substances are currently in hand at Moredun. Success could lead to an improved "Mark 2 Barbervax", possibly by 5 years' time.

7 Conclusions and recommendations

7.1 Conclusions

It was concluded that the field trials confirmed the efficacy of Barbervax as a means for controlling Barber's Pole worm in lambs and extended its use to hoggets, which, if previously immunised as lambs, required only 4 vaccine boosts to provide season long protection.

The results encouraged the view that Barbervax could be a useful additional tool to control Barber's pole worm in endemic areas of Australia.

7.2 Recommendations

It is suggested that more detailed research should be conducted to evaluate Barbervax in the following areas:

a) Currently, the recommended Barbervax immunization regime for lambs consists of 5 doses over the season starting at marking time. The first 3 doses should be given 3 to 4 weeks apart and the last two at six week intervals. More work should be done to improve this regime by reducing the number of immunizations. For example, in dry springs it should be possible to obtain season long cover by 4 doses given at marking and weaning and then two more boosts at 6 week intervals. Alternatively, after being primed by an initial vaccination at marking time, it may be possible to synchronise subsequent immunizations with the onset of rainfall and thus increased availability of infective larvae on the pasture.

b) Does it work in goats?

c) If promising preliminary trials at Moredun indicate a potential Mark 2 vaccine with a longer duration of immunity, it will need to be evaluated under on-farm conditions in Australia.

8 Bibliography

9 Appendices

9.1 Appendix 1 – Replicated plot trial with lambs at CSIRO, Arding.

CSIRO Livestock Industries

Field test of Moredun *Haemonchus* vaccine efficacy in Merino lambs where vaccinated and control lambs grazed separately on replicated plots.

Sponsor:	Name:	Julie Fitzpatrick Moredun Group Director
	Address:	Moredun Institute The Moredun Group Pentlands Science Park Bush Loan Penicuik Midlothian Scotland, UK
Sponsor Monitor and Representative:	Name:	David Smith
	Quals:	BVMS, PhD
	Address:	The Moredun Group Pentlands Science Park Bush Loan Penicuik Midlothian Scotland, UK
	Phone:	+44 (0)131 445 6131
	E-mail:	David.Smith@moredun.ac.uk
Investigator:	Name:	Malcolm Knox
	Quals:	BSc(Hons), MSc, PhD
	Address:	CSIRO Livestock Industries F.D. McMaster Laboratory-Chiswick Armidale NSW 2359
	Phone:	02 6776 1440
	E-mail:	Malcolm.Knox@csiro.au

Field test of MRI *Haemonchus* vaccine efficacy in Merino yearlings.

SUMMARY

Haemonchus contortus is the major nematode pathogen in high rainfall areas where small ruminants are produced. An effective vaccine against this pathogen is highly desirable. During the summer of 2011-2012 the efficacy of the Moredun Research Institute *Haemonchus* vaccine was assessed in 40 young "Elite" Merino lambs in the field during the period post weaning (project B.AHE.0068). Vaccinated and control lambs in that trial grazed together, but farmers would be advised never to graze unvaccinated sheep with ones which had been vaccinated. The object of present trial was to evaluate the same vaccine over the 2012-2013 summer where vaccinated and control lambs grazed separately on replicated (x4) paddocks.

The course of vaccinations stimulated a high titre antibody response from two weeks after the first booster injection. In contrast, titres in the controls were negligible though they had risen slightly by the end of the trial.

Significantly fewer *Haemonchus* eggs were shed on to the plots containing vaccinated as opposed to unvaccinated lambs and no "non-responders" were identified amongst the vaccinates. This difference was reflected in the degree of anaemia observed; blood haemoglobin concentrations being significantly lower in the controls than the vaccinates during the period of peak egg output. Furthermore, 35 of the 40 unvaccinated lambs required a precautionary drench to prevent potentially fatal anaemia, but only 2 of the 40 vaccinated lambs required such treatment.

As a result of their reduced egg output, the vaccinates enjoyed the epidemiological benefit of being exposed to significantly fewer infective larvae than the controls during the last two months of the trial. It was concluded that Barbervax could be a useful tool for controlling Barber's Pole worm.

Field test of MRI *Haemonchus* vaccine efficacy in Merino hoggets.

1. Objective

To assess the efficacy of "Barbervax", the Moredun Research Institute *Haemonchus* vaccine, when vaccinated and control Merino lambs grazed separately over the high risk period of exposure to this parasite. It was anticipated that the results would demonstrate the added epidemiological benefit of separate grazing and that they would be included in the registration package.

2. Justification

Haemonchus contortus is the major nematode pathogen in high rainfall areas of Australia where small ruminants are produced. An effective vaccine against this pathogen is highly desirable. Previous work established that Barbervax induced high levels of protection in lambs on six different Australian farms during 2010-2012, but, because vaccinated and control lambs grazed together in those trials, the potential epidemiological benefit of separate grazing was not investigated. The present study aims to address that question, by grazing vaccinates and controls apart on adjacent, replicated paddocks replicated four times.

3. Compliance

This study was conducted in accordance with the approved protocol and with CSIRO Standard Operating Procedures, unless otherwise stated, and the study objectives were achieved.

4. Test site

Animal phase
CSIRO Livestock Industries
Arding Field Station
Armidale NSW 2350

Laboratory phase
CSIRO Livestock Industries
F.D. McMaster Laboratory- Chiswick
Armidale NSW 2350

Antibody analyses
Moredun Research Institute
Edinburgh, UK

5. Study dates

Start date (Animal Phase): 5 Nov 2012
Finish date (Animal phase): 29 April 2013
Finish date (laboratory phase): 25 September 2013

6. Study design

The design was a randomised field study. Eighty lambs (40 female and 40 castrate male) were randomly selected from the same CSIRO Elite Merino flock at Arding, identified using ear tags and allocated to two equal groups on the basis of weight and sex, one vaccinated, the other unvaccinated (i.e. the controls). After the first immunisation (V1) on November 7, both groups grazed together with their mothers for four weeks, when they were weaned and introduced to 8 adjacent 0.7 ha, paddocks in such a way that each contained either 10 vaccinated or 10 control lambs. All sheep were given 4 mL Hat-Trick® (Merial Australia; abamectin 1 g/L, levamisole 33.9 g/L, oxfebdazole 22.7 g/L) and the vaccinates received their second immunisation at this time (V2). V3 was given a month later and V4 and V5 were given at 6 week intervals thereafter.

Faecal samples were collected from individual sheep at 2 week intervals from November 7 until April 24 for faecal worm egg count (FEC) estimation. On the same days, blood was collected by jugular venipuncture into 6 mL sodium heparin vacutainers for haemoglobin concentration estimation (using the Haemocue method) and for plasma collection. Liveweights were recorded at Days -2, 98 and 173 of the trial. Laboratory personnel performing FEC and haemoglobin analysis were blinded as to treatment groups.

If at any time during the trial, the blood haemoglobin concentration of any animal was <8.5 g/100mL (equivalent PCV = 25%) or the FEC was >15,000, that sheep was treated immediately with Hat-Trick® (or an alternative short-acting anthelmintic effective against *Haemonchus*) at the manufacturer's recommended dose rate. The animal remained with the trial flock after such treatment.

During the second halves of February, March and April a pair of drenched, age-matched castrate male tracer lambs from the Elite flock was introduced to each paddock for two weeks. These were removed, housed indoors for two weeks, then killed and their abomasal worm burdens determined.

7. Investigational and control products

a. Investigational Veterinary Product (IVP):

Name:	BarberVax	Batch No.:	HCD220311C-00?
Composition:	*Haemonchus* antigen and saponin adjuvant	Expiry Date:	October 2013
Dose Rate:	5µg antigen and 1mg saponin	WHP:	12 months

b. Source: WormVax Laboratory
Animal Health Laboratory
DAFWA
444 Albany Highway
Albany, W.A. 6330

c. Storage: Refrigerated at 4°C until use.

d. Safety: A MSDS was not provided by the Sponsor. The IVP was administered using a specially designed safety vaccine gun to protect against accidental injection.

e. Assays: A Certificate of Analysis was not provided for the IVP.

f. Drug Disposal: All remaining IVP was retained at CSIRO pending disposal advice from the Sponsor.

8. Treatment

a. **Treatment administration**: Vaccinations were delivered subcutaneously into the neck of the lambs using a 1 mL Simcro Securus Veterinary Injector (Simcro Animal Health Delivery Systems, New Zealand).
b. **Treatment frequency:** On five occasions at Weeks 0, 4, 8, 14 and 20.

c. **Dose**: 1 mL per lamb.

9. Schedule of events

Date	Day	Activity
5 November	-2	Identify 80 2012 drop Elite lambs (40 female, 40 castrate male) for trial and record live weights.
7 November	0	Week 0 - assign lambs to treatment groups FEC and blood samples collected. Vaccinate (V1) 40 lambs. Return to pasture with remaining Elite lambs and mothers.
12 November	5	Welfare check
15 November	8	Welfare check
21 November	14	Week 2 - FEC 80 trial Lambs
23 November	16	Welfare check
26 November	19	Welfare check
29 November	22	Welfare check
5 December	28	Week 4 - FEC and blood samples from lambs, assign to groups. Vaccinate (V2) 40 lambs. Treat all lambs with 4 mL Hat Trick® and wean to pasture plots in E Lane.
7 December	30	Welfare check
11 December	34	Welfare check
14 December	37	Welfare check
17 December	40	Welfare check
19 December	42	Week 6 - FEC and blood samples from lambs. Treat lambs with Pro-Guard® (cyromazine 500 g/L; Landmark Operations Limited) backline to prevent flystrike.
21 December	44	Welfare check
24 December	47	Welfare check
28 December	51	Welfare check
31 December	54	Welfare check
3 January	57	Week 8 - FEC and blood samples from lambs. Vaccinate (V3) 40 lambs.
7 January	61	Welfare check
10 January	64	Welfare check
14 January	68	Welfare check
16 January	70	Week 10 - FEC and blood samples from lambs
18 January	72	Welfare check

21 January	75	Welfare check
25 January	79	Welfare check
29 January	83	Welfare check
30 January	84	Week 12 - FEC and blood samples from lambs
4 February	89	Welfare check
8 February	93	Welfare check
11 February	96	Welfare check. Drench 16 Tracer 1 lambs 6 mL TriGuard®(Merial Australia; abamectin 1 g/L, levamisole 33.9 g/L, oxfebdazole 22.7 g/L), hold in yards and feed lucerne hay and water.
13 February	98	Week 14 - FEC and blood samples from lambs. Liveweights recorded. Vaccinate (V4) 40 lambs. 16 Tracer 1 lambs moved to E Lane plots (2 per plot).
14 February	99	Drench lambs 7021 and 7163 (Plot 1) with 10 mL Hat-Trick®.
15 February	100	Welfare check
18 February	103	Welfare check
19 February	104	Welfare check
22 February	107	Welfare check
25 February	110	Welfare check
27 February	112	Week 16 - FEC and blood samples from lambs. 16 Tracer 1 lambs from E Lane plots (2 per plot) to the Chiswick Animal House.
28 February	113	Drench lambs 7034, 7081, 7116, 7158, 7256, 7298, 7317 (Plot 1) with 6 mL TriGuard®.
1 March	114	Welfare check
4 March	117	Welfare check
6 March	119	Welfare check
11 March	124	Welfare check. Drench 16 Tracer 2 lambs 6 mL TriGuard®, hold in yards and feed lucerne hay and water.
13 March	126	Week 18 - FEC and blood samples from lambs. 16 Tracer 2 lambs moved to E Lane plots (2 per plot).
14 March	127	Drench lambs 7036, 7163 (Plot 1); 7284 (plot 2); 7227 (Plot 5) with 6 mL TriGuard®.
15 March	128	Welfare check
18 March	131	Welfare check
20 March	133	Welfare check
21 March	134	Welfare check

25 March	138	Welfare check
27 March	140	Week 20 - FEC and blood samples from lambs. 16 Tracer 2 lambs removed to Chiswick Animal House from E Lane plots (2 per plot).
28 March	141	Drench lambs 6 mL TriGuard®: 7034, 7081, 7116, 7158, 7256, 7298, 7317 (Plot 1); 7003, 7039, 7046, 7066, 7099, 7168, 7199, 7271, 7280 (Plot 2); 7215 (Plot 3); 7093 (Plot 4); 7023, 7064, 7103, 7148, 7205, 7209, 7265 (Plot 5); 7009, 7019, 7027, 7076, 7192, 7200, 7222, 7279, 7304 (Plot 6).
2 April	146	Welfare check
5 April	149	Welfare check
8 April	152	Welfare check. Drench 16 Tracer 3 lambs 6 mL TriGuard®, hold in yards and feed lucerne hay and water.
10 April	154	16 Tracer 3 lambs moved to E Lane plots (2 per plot)
11 April	155	Week 22 - FEC and blood samples from lambs.
12 April	156	Drench lambs 6 mL TriGuard®: 7163 (Plot 1); 7284 (Plot 2); 7115, 7205, 7227, 7233 (Plot 5).
15 April	159	Welfare check
17 April	161	Welfare check
19 April	163	Welfare check
22 April	166	Welfare check
24 April	168	Week 24 - FEC and blood samples from lambs. 16 Tracer 3 lambs removed to Chiswick Animal House from E Lane plots (2 per plot). All remaining trial lambs drenched 4 mL Zolvix (monepantel and conc).
29 April	173	Lamb liveweights recorded. Lambs returned to Elite lamb flock.

10. Animal Management

a. **Animal Welfare:** Study animals were managed similarly and with due regard for their welfare. Animals were observed at least twice weekly for health problems according to AEC requirements. Animals were handled in compliance with CSIRO AEC 12/25 approved 18/10/12, and any applicable local regulations.

b. **Health Management:** Any health problems or adverse events that occurred during the study were recorded (see Study schedule above).

c. **Housing:** Routine management practices were followed. All trial animals had *ad-lib* access to pasture consisting of rye, *Phalaris*, clover and native grass species. Potable water was supplied *ad-lib*.

d. **Animal disposal:** All remaining animals were returned to the CSIRO flocks on Arding at the conclusion of the study.

11. Study Procedures

a. **Trial Log:** All scheduled and unscheduled events during the study were recorded.

b. **Plasma Sample Storage, Transfer and Disposal:** Replicate 1 and 2 samples were stored in separate temperature logged freezers at approximately -20°C until delivery on ice-bricks to Veterinary Health Research, Armidale for onward dispatch to the Moredun Research Institute, Edinburgh. Replicate 2 plasma samples will be held in frozen storage (-20°C) at CSIRO until disposal is approved by the study sponsor.

12. Assessment of Effects

a. **Sheep live weights**: Animals were weighed at Days -2, 98 and 173 and individual animal live weights were recorded. Animal weigh scales were checked pre- and post-weighing with calibrated test weights. Live weights and liveweight change during the study were compared between groups to determine treatment effects, if any, and are detailed in the results section of this report.

b. **Haemoglobin concentration**: Blood haemoglobin concentration from individual lamb whole blood was measured using the Hemocue 201 Hb Analyser. Changes during the study were compared between groups to determine treatment effects, if any, and are detailed in the results section of this report.

c. **Faecal worm egg counts and larval differentiations**: Faecal samples were collected at two week intervals, labelled with the animal ID and individual worm egg counts and group bulk larval differentiation were performed. Faecal worm egg counts and larval differentiation were compared to determine treatment effects, if any, and are detailed in the results section of this report.

   Note: where an animal received a salvage drench at any point throughout the study, the subsequent FEC sample collected within 14 days of the salvage drench was excluded from group mean FEC calculations.

d. **Blood antibody analyses**: Blood samples were processed for collection of plasma samples on the day of collection. Samples were individually labelled with the study number, animal number, study date and day, sample type. Frozen (-20°C) plasma samples were forwarded to the Moredun Research Institute for anti-vaccine antibody titre analysis at completion of the study. Results of analyses were compared to determine treatment effects, if any, and are detailed in the results section of this report.

13. Statistical Analyses

Faecal egg count data obtained from an individual lamb 14 days after it had been given a precautionary drench were ignored, but subsequent data were re-included in the analysis.

Faecal egg counts, blood haemoglobin concentrations and bodyweights were compared between groups by the t test. The faecal egg counts were log transformed prior to analysis.

14. Results

a. **Deaths**

No sheep died during the trial.

b. ***Haemonchus*** **Egg Counts**

When averaged over the trial, mean *Haemonchus* faecal egg counts of the lambs in the vaccinated plots were significantly ($p<0.01$) lower than those grazing the control plots (Fig 1a and 4a). Examination of the overall trial average counts of each vaccinated lamb (Fig 1b) did not reveal any "non-responders", defined as a vaccinate with a mean egg count greater than the lower 95% confidence limit of the control group. No significant differences were detected in mean *Haemonchus* faecal egg counts between plots within either the vaccinated or control groups, but the difference between these treatment groups was highly significant (Fig 1c and Table A 4 in Appendix 4 below).

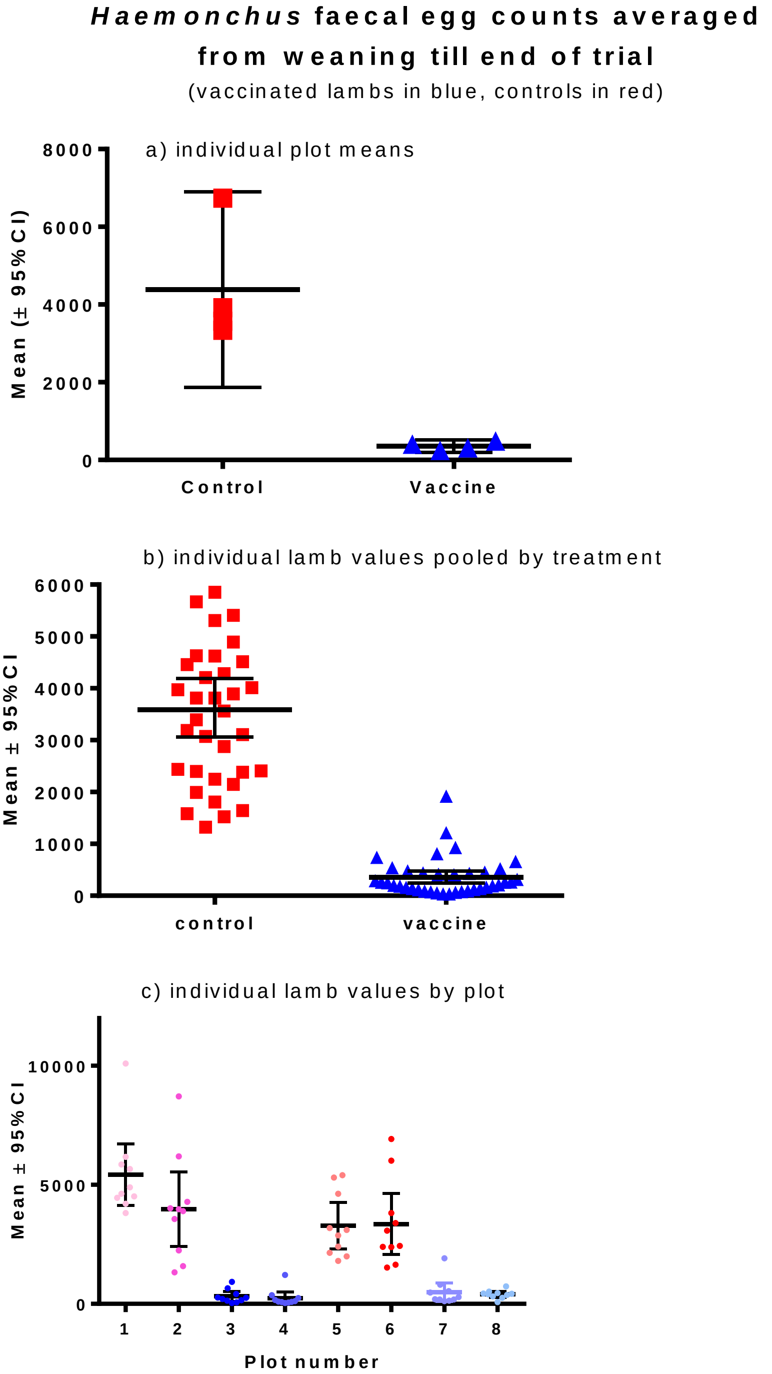


Fig 1. Mean *Haemonchus* egg counts from weaning to the end of trial.

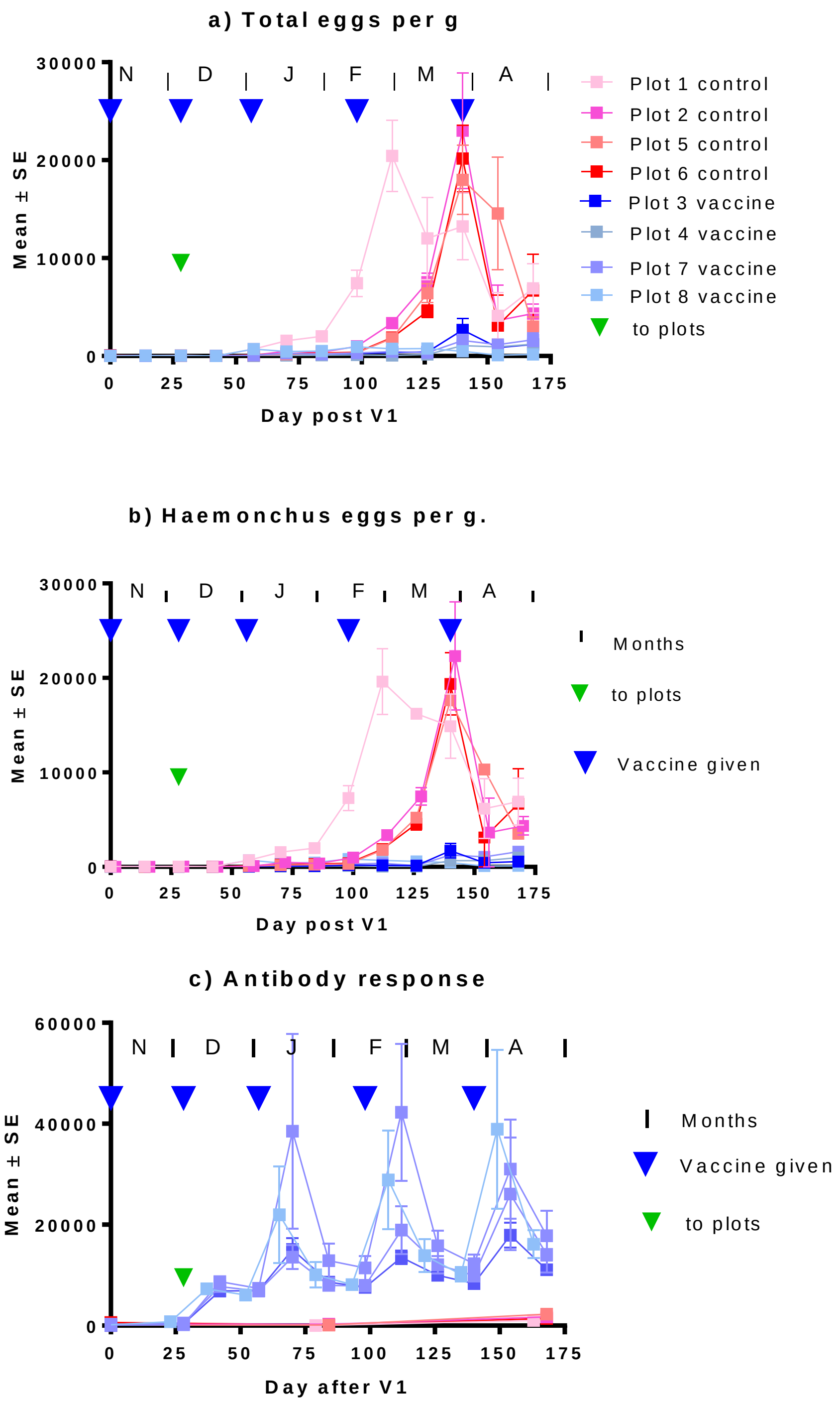


Fig 2. Kinetics of total and *Haemonchus* specific egg counts and the anti-vaccine antibody response in each plot. Note: control plots in shades of red, vaccinated plots in shades of blue; green triangle denotes lamb placement in trial paddocks.

Haemonchus was always the dominant nematode genus cultured from the faecal samples (see Table A6 in Appendix 1 for full details). During November, December and January only modest numbers of eggs were detected in all sheep, but from February onwards mean egg counts increased dramatically in the four control plots, peaking around 20,000 per g in every case (Fig 2 b). In contrast the peak mean count in all four vaccinated plots was less than 1,800 eggs per g.

Little in the way of an anti-vaccine antibody response was detected in the control lambs, though their titres at the end of the trial were slightly raised compared to those at the start (Fig 2c). In contrast, the vaccinated lambs showed significant serum antibody responses from two weeks after the second immunisation. Although there was considerable individual variation, clear spikes in the response were observed after the second and subsequent boosts (Fig 2c).

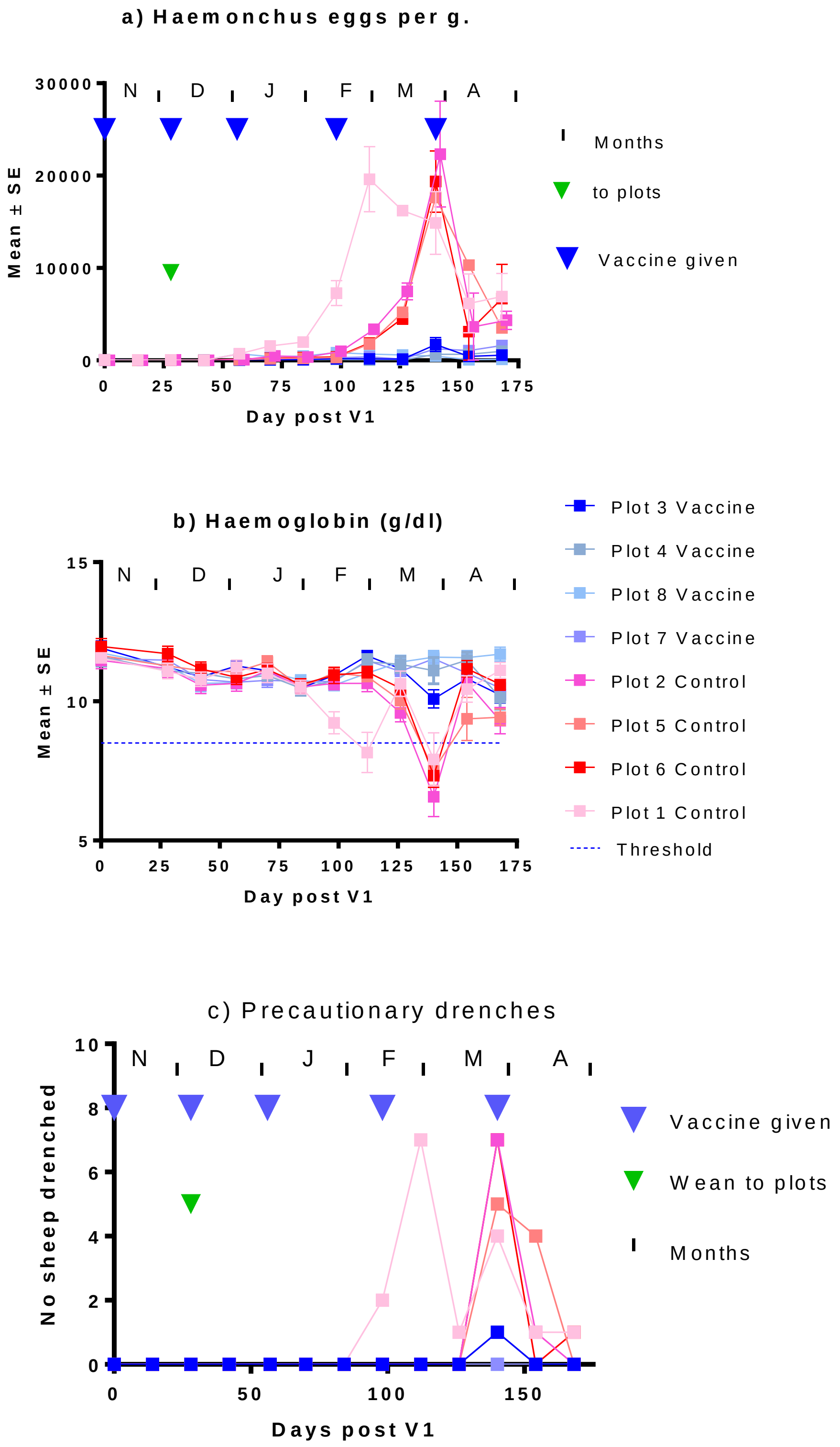
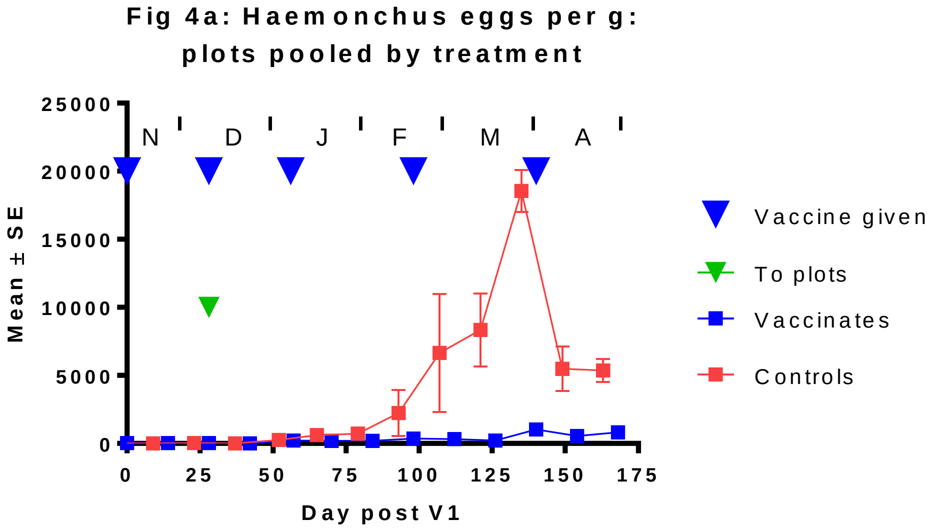


Fig 3. Kinetics of *Haemonchus* specific egg counts in relation to blood haemoglobin concentrations and the number of precautionary drenches given in each plot. Note: control plots in shades of red, vaccinated plots in shades of blue; green triangle denotes lamb placement in trial paddocks.

For reasons not understood, the mean egg counts in plot 4 started to rise about 4 weeks earlier than the other 3 control plots and this was reflected in an earlier dip in blood haemoglobin followed by precautionary drenching, which restored haemoglobin to values close to normal (Fig 3 a, b and c).

A total of 41 drenches were given to 35 of the 40 control lambs in response to their egg counts exceeding 10,000 per g or their blood haemoglobin falling below the pre-set threshold of 8.5 g/dl. In contrast, only two vaccinated lambs needed anthelmintic support, each requiring a single drench on Day 140.



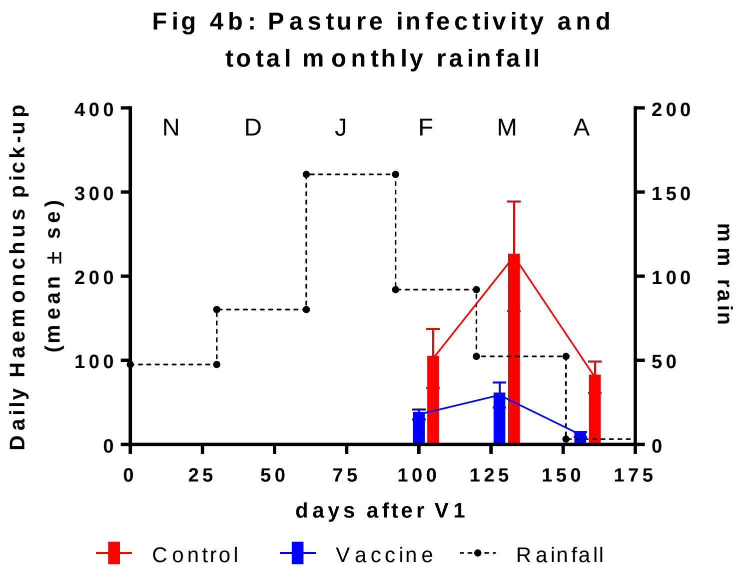


Fig 4. Kinetics of the infectivity of vaccine and control plots relative to the number of eggs being deposited on the pasture and the monthly rainfall.

During February a mean of about 100 new *Haemonchus* were establishing in the tracer lambs on the control plots each day. During March, this figure more than doubled, but fell back during April (Fig 4b). These changes were ascribed to much increased deposition of eggs during March (Fig 4a) and the lack of rain in April (Fig 4b), respectively. A similar but less pronounced trend occurred in the vaccinated plots.

During March and April significantly fewer *Haemonchus* were acquired by the tracer sheep grazing the vaccinate plots compared to the control plots (p<0.05).

c. Bodyweights.

The sheep were weighed on days -2, 98 and 173, (ie at the beginning, the middle and end of the trial). Group mean bodyweights are presented in Figure 5 below. No significant differences (p<0.05, by analysis of variance) were detected between the vaccinated and control groups at any stage of trial (see foot of Table A.1 in Appendix 1 for the results of the analysis).

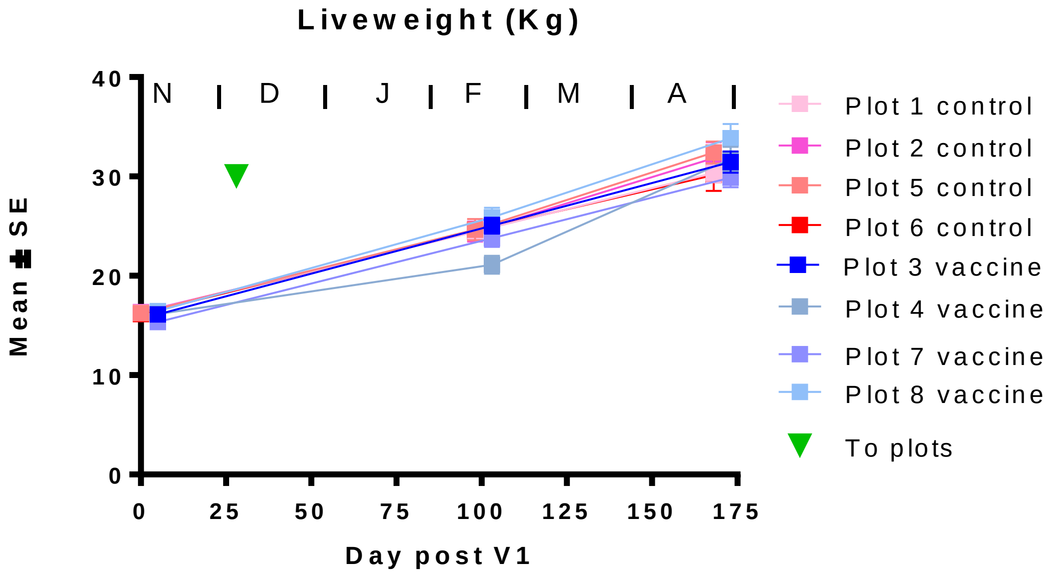


Fig . Sheep weights during the course of the trial

15. Discussion/Conclusions

It was very clear that the course of Barbervax maintained high circulating titres of anti-vaccine antibodies after the first boost. These high titres greatly suppressed a) the surge of *Haemonchus* egg outputs observed in every control plot during February or March b) the degree of anaemia recorded in the control lambs and c) significantly reduced pasture contamination during March and April.

Since immunisation did not adversely affect the growth rate of the lambs, it seems reasonable to conclude that Barbervax has significant potential for controlling Haemonchosis in lambs

Appendix 1. Tabulated and raw data

Table A.1: Allocation of trial lambs to the treatment groups and plots and live weights (kg) on Days -2, 98 and 173 of the trial.

Tag	Group	Sex	Rep	Plot	05/11/2012	13/02/2013	29/04/2013
7021	Control	Male	4	1	15.5	24.5	32.5
7034	Control	Male	4	1	17	28	34.5
7036	Control	Male	4	1	19	28	34
7081	Control	Male	4	1	17.5	24	29
7116	Control	Male	4	1	15.5	23	28.5
7158	Control	Female	4	1	18.5	24.5	29.5
7163	Control	Female	4	1	17.5	23	30.5
7256	Control	Female	4	1	12.5	20	26.5
7298	Control	Female	4	1	14.5	22.5	26
7317	Control	Female	4	1	15.5	27	32.5
7003	Control	Male	1	2	13.5	23	31
7039	Control	Male	1	2	18	24	34
7046	Control	Male	1	2	17	24	34
7066	Control	Male	1	2	19	28.5	37.5
7099	Control	Male	1	2	16.5	28	38
7168	Control	Female	1	2	13.5	22	28
7199	Control	Female	1	2	15	18	21
7271	Control	Female	1	2	18	27.5	33
7280	Control	Female	1	2	17.5	25	33.5
7284	Control	Female	1	2	15.5	24.5	28.5
7007	Vaccine	Male	1	3	19	28	35.5
7049	Vaccine	Male	1	3	17	26.5	34.5
7086	Vaccine	Male	1	3	14.5	23.5	29.5
7113	Vaccine	Male	1	3	17.5	28	35.5
7129	Vaccine	Male	1	3	16	23	24.5
7215	Vaccine	Female	1	3	17	26	30.5
7226	Vaccine	Female	1	3	18	27.5	33.5
7263	Vaccine	Female	1	3	14.5	23.5	30.5
7270	Vaccine	Female	1	3	15	24	31
7272	Vaccine	Female	1	3	12.5	20.5	29.5
7055	Vaccine	Male	2	4	15	21	32
7092	Vaccine	Male	2	4	19.5	24.5	36.5
7093	Vaccine	Male	2	4	15.5	20.5	33
7138	Vaccine	Male	2	4	16.5	21	31
7155	Vaccine	Male	2	4	17	26	39
7174	Vaccine	Female	2	4	16.5	19.5	28
7177	Vaccine	Female	2	4	18.5	20.5	31.5

7218	Vaccine	Female	2	4	15.5	23	32
7219	Vaccine	Female	2	4	15	19.5	28.5
7302	Vaccine	Female	2	4	12.5	15.5	26
7023	Control	Male	2	5	18.5	28.5	34.5
7064	Control	Male	2	5	14.5	24.5	31.5
7103	Control	Male	2	5	18	28.5	37
7115	Control	Male	2	5	15.5	26	34
7148	Control	Male	2	5	16.5	21.5	34.5
7205	Control	Female	2	5	14.5	22	26.5
7209	Control	Female	2	5	15.5	23.5	31
7227	Control	Female	2	5	20	28	35.5
7233	Control	Female	2	5	16.5	25.5	32.5
7265	Control	Female	2	5	13	18.5	27
7009	Control	Male	3	6	18.5	29.5	39
7019	Control	Male	3	6	15.5	26.5	35.5
7027	Control	Male	3	6	17	21.5	29
7076	Control	Male	3	6	17.5	23.5	30.5
7110	Control	Male	3	6	16.5	27	30.5
7192	Control	Female	3	6	15.5	24	31
7200	Control	Female	3	6	14	22	20
7222	Control	Female	3	6	16.5	22.5	26.5
7279	Control	Female	3	6	12	22.5	30.5
7304	Control	Female	3	6	18.5	26.5	29
7033	Vaccine	Male	3	7	17	27	33.5
7061	Vaccine	Male	3	7	18.5	26.5	31.5
7084	Vaccine	Male	3	7	13	19.5	28
7141	Vaccine	Male	3	7	18	27.5	35.5
7149	Vaccine	Male	3	7	15.5	25.5	30
7159	Vaccine	Female	3	7	14	24	31
7164	Vaccine	Female	3	7	13	22.5	29
7216	Vaccine	Female	3	7	15.5	22.5	28.5
7258	Vaccine	Female	3	7	12	20.5	24.5
7300	Vaccine	Female	3	7	17	22.5	27.5
7032	Vaccine	Male	4	8	17	27.5	36
7038	Vaccine	Male	4	8	15.5	24	27.5
7107	Vaccine	Male	4	8	17.5	28	36.5
7117	Vaccine	Male	4	8	16.5	24.5	33
7146	Vaccine	Male	4	8	18	24.5	34
7182	Vaccine	Female	4	8	16	25	34.5
7201	Vaccine	Female	4	8	13.5	20.5	25.5
7238	Vaccine	Female	4	8	18.5	31	41
7295	Vaccine	Female	4	8	14.5	24.5	33
7318	Vaccine	Female	4	8	17.5	29.5	37.5

The results of an analysis of variance of mean control and vaccine plot live weights on days 0, 98 and 168 (start, middle and end of the trial) are shown below. No significant differences were detected at any stage.

Number of families	1
Number of comparisons per family	3
Alpha	0.05

Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Control 0 vs. Vaccine 0	0.2500	-1.963 to 2.463	**No**	**ns**
Control 98 vs. Vaccine 98	0.5625	-1.651 to 2.776	**No**	**ns**
Control 168 vs. Vaccine 168	-0.5500	-2.763 to 1.663	**No**	**ns**

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	t	DF
Control 0 vs. Vaccine 0	16.26	16.01	0.2500	0.8412	4	4	0.2972	18
Control 98 vs. Vaccine 98	24.53	23.96	0.5625	0.8412	4	4	0.6687	18
Control 168 vs. Vaccine 168	31.19	31.74	-0.5500	0.8412	4	4	0.6539	18

Table A.2 Haemoglobin concentration (mg/mL) for trial lambs. Those below the 85 mg/ml threshold are highlighted in yellow, those drenched because their egg counts were > 10,000 / g are highlighted in pink.

Tag	Grp	Plot	7/11/12	5/12/12	19/12/12	3/1/13	16/1/13	30/1/13	13/2/13	27/2/13	13/3/13	27/3/13	10/4/13	24/4/13
7021	C	1	112	107	105	106	101	91	85	122	120	116	103	94
7034	C	1	121	113	106	115	108	105	89	58	109	73	115	113
7036	C	1	111	119	117	119	122	119	113	99	90	124	108	109
7081	C	1	112	100	103	96	93	104	86	61	105	49	105	118
7116	C	1	121	127	117	117	121	112	102	85	121	64	119	113
7158	C	1	107	107	106	107	95	100	97	75	105	57	96	115
7163	C	1	117	104	110	116	114	99	66	109	74	112	68	114
7256	C	1	115	107	96	113	119	108	94	78	115	85	121	109
7298	C	1	123	120	121	124	118	111	99	80	114	77	107	100
7317	C	1	117	104	98	110	109	102	92	49	110	34	102	126
7003	C	2	129	126	119	124	125	118	119	128	120	91	132	68
7039	C	2	111	125	113	109	107	101	98	104	102	72	117	109
7046	C	2	113	109	103	99	113	101	114	99	99	80	100	88
7066	C	2	117	120	97	94	106	101	101	101	87	64	109	87
7099	C	2	107	105	96	99	109	104	102	106	90	65	103	105
7168	C	2	108	94	93	100	101	95	105	103	95	45	99	85
7199	C	2	126	111	105	108	117	108	103	101	92	51	114	105
7271	C	2	119	113	119	117	113	111	116	115	95	41	105	114
7280	C	2	120	108	105	107	110	111	109	111	95	40	109	95
7284	C	2	97	107	108	108	109	101	98	97	84	108	80	74
7007	V	3	131	116	117	115	104	109	111	120	116	119	110	91
7049	V	3	131	113	117	116	111	103	110	115	119	98	100	120

7086	V	3	108	108	104	106	101	94	97	116	101	95	101	99
7113	V	3	120	114	115	109	110	101	114	119	114	109	112	108
7129	V	3	122	120	103	112	109	102	99	104	107	99	104	105
7215	V	3	124	114	112	113	121	112	110	124	109	79	120	85
7226	V	3	118	101	111	115	114	118	117	119	110	101	110	104
7263	V	3	114	113	104	108	113	102	105	109	108	104	100	101
7270	V	3	116	126	112	123	119	105	120	122	122	106	114	102
7272	V	3	107	98	93	110	108	102	110	116	111	99	110	108
7055	V	4	96	97	99	93	105	94	99	102	96	100	99	106
7092	V	4	116	106	113	112	115	99	105	119	114	114	124	102
7093	V	4	109	112	114	111	109	109	105	111	105	74	111	102
7138	V	4	123	114	113	114	108	107	112	123	115	115	118	110
7155	V	4	124	118	111	112	110	106	111	124	123	114	120	78
7174	V	4	103	106	108	108	110	105	105	113	113	116	120	125
7177	V	4	131	115	113	109	109	103	105	116	107	119	114	89
7218	V	4	126	112	103	100	99	97	112	99	112	114	106	111
7219	V	4	116	113	105	104	106	124	112	121	124	114	107	93
7302	V	4	117	116	122	117	122	103	109	114	124	130	129	97
7023	C	5	107	99	106	108	113	107	120	111	94	39	98	116
7064	C	5	125	124	127	125	129	115	115	113	112	67	117	89
7103	C	5	122	106	105	106	109	97	113	111	101	84	109	85
7115	C	5	121	123	127	120	117	113	113	115	112	90	74	86
7148	C	5	112	111	100	108	110	105	101	107	96	69	116	102
7205	C	5	103	108	103	107	109	97	102	98	96	65	43	91
7209	C	5	125	118	111	110	114	105	110	116	106	67	112	89
7227	C	5	122	126	119	117	123	108	101	99	79	102	72	92

7233	C	5	114	109	109	101	110	105	115	107	101	90	85	94
7265	C	5	114	103	103	104	107	98	112	113	106	81	111	99
7009	C	6	112	118	112	102	103	98	107	105	98	69	108	112
7019	C	6	112	116	108	112	110	106	103	107	112	71	119	101
7027	C	6	128	116	110	104	110	107	111	110	113	82	115	96
7076	C	6	132	127	118	112	115	106	107	111	100	63	114	109
7110	C	6	131	110	123	114	119	108	118	119	105	90	95	97
7192	C	6	128	132	118	119	124	117	117	115	111	80	126	114
7200	C	6	108	103	95	95	102	94	93	106	96	59	103	102
7222	C	6	110	115	105	103	112	112	119	121	113	93	121	116
7279	C	6	121	125	118	121	115	110	120	111	94	51	112	108
7304	C	6	115	109	109	104	105	102	99	100	105	75	104	106
7033	V	7	106	107	99	104	110	115	103	114	112	114	108	105
7061	V	7	115	112	104	112	107	114	112	117	110	121	116	110
7084	V	7	113	112	111	101	105	105	110	112	108	104	99	92
7141	V	7	137	127	122	119	117	108	108	116	112	121	120	111
7149	V	7	113	113	108	108	108	107	109	114	101	115	110	103
7159	V	7	114	116	118	114	119	111	114	116	117	121	118	104
7164	V	7	112	109	108	110	108	102	104	113	111	118	114	108
7216	V	7	113	128	107	107	111	110	111	130	119	125	111	117
7257	V	7	110	106	99	93	91	95	102	101	106	109	102	100
7300	V	7	121	118	103	101	99	105	98	113	112	105	103	98
7032	V	8	125	124	111	110	106	108	105	112	121	115	111	121
7038	V	8	131	114	103	102	109	113	103	102	100	109	107	109
7107	V	8	112	103	99	103	106	109	115	111	124	120	111	113
7117	V	8	114	110	106	107	120	105	106	111	116	113	123	124

7146	V	8	118	96	91	95	92	98	96	111	114	110	115	108
7182	V	8	121	109	101	103	104	114	99	108	114	124	124	121
7201	V	8	103	107	104	106	113	110	114	109	114	107	104	113
7238	V	8	117	125	120	115	129	105	110	110	114	128	124	129
7295	V	8	123	119	123	120	115	108	109	116	121	125	127	125
7318	V	8	110	120	107	106	111	107	101	111	103	108	111	107

Table A.3 Faecal worm egg counts (eggs/g faeces) for trial lambs.

Precautionary drenches during the trial : Hb <85mg/mL yellow background, or FEC>15000 pink background.

			Days after V1.												
			0	14	28	42	57	70	84	98	112	126	140	154	168
Tag	**Gp**	**Plot**	**07/11/12**	**21/11/12**	**05/12/12**	**19/12/12**	**03/01/13**	**16/01/13**	**30/01/13**	**13/02/13**	**27/02/13**	**13/03/13**	**27/03/13**	**10/04/13**	**24/04/13**
7021	C	1	100	0	0	0	1600	3600	2700	12800	0	0	100	4900	25600
7034	C	1	0	0	0	0	600	1500	2400	8500	19200	0	14900	0	2800
7036	C	1	0	0	0	0	100	300	200	3100	11400	16200	0	4600	9200
7081	C	1	0	100	0	0	1800	4300	4800	9600	41200	0	22500	0	8600
7116	C	1	0	100	0	0	0	1500	1600	5800	14500	0	30000	0	2900
7158	C	1	0	0	0	0	600	700	1400	2400	8100	0	15900	0	5600
7163	C	1	200	0	400	0	200	600	1400	15000	0	7800	0	15100	0
7256	C	1	100	0	0	0	0	0	200	2800	26400	0	8400	0	1200
7298	C	1	0	100	0	0	700	200	2400	6000	23400	0	6600	0	2400
7317	C	1	0	0	0	0	1600	2800	3100	8300	19200	0	20700	0	3800
7003	C	2	100	0	0	0	0	1100	400	900	4000	10000	20700	0	2600
7039	C	2	0	0	0	0	200	700	300	900	2600	4800	22000	0	9400
7046	C	2	0	0	0	0	100	500	500	1200	1400	2400	6300	0	2400
7066	C	2	0	100	0	0	200	900	1200	1400	3200	10000	21000	0	5800
7099	C	2	0	0	100	0	0	800	300	900	3200	4400	7800	0	200
7168	C	2	0	0	0	0	200	100	300	1000	3900	9400	21200	0	4400
7199	C	2	100	100	100	0	100	200	300	1300	3800	7600	45400	0	4600
7271	C	2	0	0	100	0	0	400	300	900	5600	11200	62900	0	7800
7280	C	2	0	0	0	0	0	0	200	1100	4000	6600	22700	0	1800

7284	C	2	0	0	0	0	100	200	100	300	2000	9000	0	10900	0
7007	V	3	100	200	0	0	0	100	0	200	0	200	1100	600	400
7049	V	3	100	0	0	0	0	0	0	200	300	800	5000	2900	4000
7086	V	3	0	0	0	0	0	200	200	700	600	300	2300	2000	1800
7113	V	3	0	0	0	0	0	200	100	0	200	200	1500	1500	1500
7129	V	3	0	100	0	0	0	0	200	300	300	100	900	500	800
7215	V	3	0	0	0	0	0	100	200	500	800	1800	12500	0	1200
7226	V	3	200	100	0	0	0	200	200	400	500	100	2100	400	1000
7263	V	3	0	0	0	0	0	100	0	0	100	200	500	100	300
7270	V	3	200	0	100	0	400	300	400	200	600	200	600	500	300
7272	V	3	0	0	0	0	0	0	0	0	100	100	0	0	600
7055	V	4	0	0	0	0	0	0	0	100	0	100	200	200	700
7092	V	4	0	0	0	0	0	0	0	100	100	100	300	400	200
7093	V	4	0	0	0	0	0	100	0	0	100	100	1300	0	0
7138	V	4	0	0	0	0	0	200	0	0	100	300	800	600	700
7155	V	4	0	100	0	0	0	200	200	200	0	200	1700	1400	1700
7174	V	4	0	0	0	0	0	0	100	0	0	100	200	100	300
7177	V	4	0	0	0	0	0	0	100	0	0	100	0	0	300
7218	V	4	0	0	100	0	0	0	100	0	0	100	500	400	600
7219	V	4	100	0	200	0	200	0	0	500	200	500	4900	5100	6800
7302	V	4	0	0	100	0	0	0	400	300	200	100	600	1200	800
7023	C	5	0	0	0	0	0	100	700	300	2900	7600	38200	0	6600
7064	C	5	0	0	0	0	300	300	200	200	2100	6200	19400	0	1700
7103	C	5	0	100	100	0	300	300	200	600	1600	3800	14600	0	3200
7115	C	5	200	0	0	0	100	0	200	200	1900	6400	13100	26400	0
7148	C	5	0	0	0	0	0	100	300	400	1800	5600	19000	0	6200

7205	C	5	0	0	0	0	100	600	500	800	3200	10400	35000	34800	0
7209	C	5	100	0	100	0	0	100	100	100	100	3000	15400	0	1900
7227	C	5	0	0	0	0	0	200	0	400	2400	12800	0	11000	0
7233	C	5	0	100	0	0	100	100	100	300	900	4000	11700	15200	0
7265	C	5	0	0	0	0	0	0	200	100	1300	4400	13500	0	1500
7009	C	6	0	0	0	0	200	600	600	400	2400	4000	15200	0	1600
7019	C	6	0	0	100	0	0	0	100	200	1000	2400	16700	0	4000
7027	C	6	0	0	0	0	200	0	100	100	1100	4800	15900	0	2400
7076	C	6	0	0	0	0	0	0	100	0	2400	6000	21900	0	4400
7110	C	6	0	100	0	0	0	200	300	100	1400	4200	11700	9300	39400
7192	C	6	400	0	100	0	200	400	200	700	2300	2600	11600	0	400
7200	C	6	0	0	100	0	300	200	300	500	2400	3600	21800	0	2400
7222	C	6	100	0	0	0	100	200	100	600	2300	6600	27500	0	1800
7279	C	6	0	0	0	0	400	600	800	1200	2700	9000	47400	0	9000
7304	C	6	100	0	0	0	0	100	400	100	800	2300	12000	0	1500
7033	V	7	0	0	200	0	100	100	100	200	200	200	400	700	100
7061	V	7	0	0	0	0	0	100	100	0	200	200	900	700	300
7084	V	7	0	0	0	0	0	0	0	200	0	400	2500	2700	4200
7141	V	7	0	0	0	0	0	0	0	100	200	200	300	600	100
7149	V	7	0	0	100	0	0	100	100	600	200	700	2400	600	1500
7159	V	7	0	0	0	0	0	200	600	400	900	300	1600	1500	1300
7164	V	7	0	0	100	0	0	0	0	0	100	0	500	700	1000
7216	V	7	0	0	0	0	0	100	200	100	600	300	600	1100	700
7257	V	7	0	0	0	0	0	0	0	100	300	400	300	200	600
7300	V	7	0	0	0	0	700	2200	700	1400	2000	600	6400	2900	6800
7032	V	8	100	0	0	0	1600	600	800	1100	600	2000	1200	500	800

7038	V	8	0	0	0	0	200	900	700	400	400	400	100	100	0
7107	V	8	0	353	100	0	0	100	100	200	400	0	0	0	0
7117	V	8	0	0	0	0	500	300	300	400	1600	800	1100	100	100
7146	V	8	0	0	0	0	200	200	700	2200	600	800	200	100	0
7182	V	8	100	0	200	0	1900	600	600	900	800	0	100	0	0
7201	V	8	0	0	0	0	200	200	400	400	300	900	1100	100	300
7238	V	8	0	0	0	0	200	200	900	1300	1200	1800	800	0	100
7295	V	8	0	0	100	0	600	600	300	1300	900	500	100	0	200
7318	V	8	0	0	0	0	1700	900	400	1200	500	500	200	0	0

Table A 4.

Mean *Haemonchus* egg counts (log transformed) of each lamb over the duration of the trial.

The title of each column shows the plot number and whether the lambs in it were vaccinated or not.

Vaccine 3	Vaccine 4	Vaccine 7	Vaccine 8	Control 1	Control 2	Control 5	Control 6
2.136721	1.934498	2.255272	2.867467	3.753277	3.590173	3.733037	3.387212
2.816241	1.869232	2.276462	2.4133	3.689398	3.603577	3.458789	3.377124
2.61595	1.959041	2.906873	1.880814	3.64914	3.120574	3.331427	3.379487
2.4133	2.214844	2.045323	2.631444	4.004149	3.631748	3.664924	3.530456
2.198657	2.561101	2.679428	2.605305	3.790637	3.198657	3.503382	3.779019
2.96708	1.69897	2.729165	2.642465	3.581039	3.599118	3.724849	3.214844
2.418301	1.50515	2.296665	2.494155	3.665112	3.791901	3.299071	3.487563
1.80618	2.071882	2.456366	2.71433	3.624179	3.940317	3.381296	3.580925
2.311754	3.084219	2.139879	2.575188	3.654273	3.551816	3.492062	3.840106
1.518514	2.392697	3.282396	2.655138	3.767156	3.35199	3.256477	3.181844

Analysis of variance of the data above.

Table Analyzed	Transform of Hc epgs				
ANOVA summary					
F	43.05				
P value	< 0.0001				
P value summary	****				
Are differences among means statistically significant? (P < 0.05)	Yes				
R square	0.8071				
Brown-Forsythe test					
F (DFn, DFd)	2.091 (7, 72)				
P value	0.0554				
P value summary	ns				
Significantly different standard deviations? (P < 0.05)	No				
Bartlett's test					
Bartlett's statistic (corrected)	22.98				
P value	0.0017				
P value summary	**				
Significantly different standard deviations? (P < 0.05)	Yes				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	29.24	7	4.177	F (7, 72) = 43.05	P < 0.0001
Residual (within columns)	6.987	72	0.09704		
Total	36.23	79			
Data summary					

Number of treatments (columns)	8
Number of values (total)	80

Comparison between treatments and within treatments by plot

Number of families	1
Number of comparisons per family	28
Alpha	0.05

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
Vaccine 3 vs. Vaccine 4	0.1911	-0.2438 to 0.6260	No	ns
Vaccine 3 vs. Vaccine 7	-0.1865	-0.6214 to 0.2484	No	ns
Vaccine 3 vs. Vaccine 8	-0.2277	-0.6626 to 0.2072	No	ns
Vaccine 3 vs. Control 1	-1.398	-1.832 to -0.9627	Yes	****
Vaccine 3 vs. Control 2	-1.218	-1.653 to -0.7828	Yes	****
Vaccine 3 vs. Control 5	-1.164	-1.599 to -0.7294	Yes	****
Vaccine 3 vs. Control 6	-1.156	-1.590 to -0.7207	Yes	****
Vaccine 4 vs. Vaccine 7	-0.3776	-0.8125 to 0.05728	No	ns
Vaccine 4 vs. Vaccine 8	-0.4188	-0.8537 to 0.01611	No	ns
Vaccine 4 vs. Control 1	-1.589	-2.024 to -1.154	Yes	****
Vaccine 4 vs. Control 2	-1.409	-1.844 to -0.9739	Yes	****
Vaccine 4 vs. Control 5	-1.355	-1.790 to -0.9205	Yes	****
Vaccine 4 vs. Control 6	-1.347	-1.782 to -0.9118	Yes	****
Vaccine 7 vs. Vaccine 8	-0.04118	-0.4761 to 0.3937	No	ns
Vaccine 7 vs. Control 1	-1.211	-1.646 to -0.7761	Yes	****

Vaccine 7 vs. Control 2	-1.031	-1.466 to -0.5963	Yes	****
Vaccine 7 vs. Control 5	-0.9777	-1.413 to -0.5428	Yes	****
Vaccine 7 vs. Control 6	-0.9691	-1.404 to -0.5342	Yes	****
Vaccine 8 vs. Control 1	-1.170	-1.605 to -0.7350	Yes	****
Vaccine 8 vs. Control 2	-0.9900	-1.425 to -0.5551	Yes	****
Vaccine 8 vs. Control 5	-0.9366	-1.371 to -0.5017	Yes	****
Vaccine 8 vs. Control 6	-0.9279	-1.363 to -0.4930	Yes	****
Control 1 vs. Control 2	0.1798	-0.2551 to 0.6148	No	ns
Control 1 vs. Control 5	0.2333	-0.2016 to 0.6682	No	ns
Control 1 vs. Control 6	0.2420	-0.1929 to 0.6769	No	ns
Control 2 vs. Control 5	0.05346	-0.3814 to 0.4884	No	ns
Control 2 vs. Control 6	0.06213	-0.3728 to 0.4970	No	ns
Control 5 vs. Control 6	0.008674	-0.4262 to 0.4436	No	ns

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Vaccine 3 vs. Vaccine 4	2.320	2.129	0.1911	0.1393	10	10	1.940	72
Vaccine 3 vs. Vaccine 7	2.320	2.507	-0.1865	0.1393	10	10	1.893	72
Vaccine 3 vs. Vaccine 8	2.320	2.548	-0.2277	0.1393	10	10	2.311	72
Vaccine 3 vs. Control 1	2.320	3.718	-1.398	0.1393	10	10	14.19	72
Vaccine 3 vs. Control 2	2.320	3.538	-1.218	0.1393	10	10	12.36	72
Vaccine 3 vs. Control 5	2.320	3.485	-1.164	0.1393	10	10	11.82	72
Vaccine 3 vs. Control 6	2.320	3.476	-1.156	0.1393	10	10	11.73	72
Vaccine 4 vs. Vaccine 7	2.129	2.507	-0.3776	0.1393	10	10	3.833	72
Vaccine 4 vs. Vaccine 8	2.129	2.548	-0.4188	0.1393	10	10	4.251	72
Vaccine 4 vs. Control 1	2.129	3.718	-1.589	0.1393	10	10	16.13	72
Vaccine 4 vs. Control 2	2.129	3.538	-1.409	0.1393	10	10	14.30	72

Vaccine 4 vs. Control 5	2.129	3.485	-1.355	0.1393	10	10	13.76	72
Vaccine 4 vs. Control 6	2.129	3.476	-1.347	0.1393	10	10	13.67	72
Vaccine 7 vs. Vaccine 8	2.507	2.548	-0.04118	0.1393	10	10	0.4180	72
Vaccine 7 vs. Control 1	2.507	3.718	-1.211	0.1393	10	10	12.29	72
Vaccine 7 vs. Control 2	2.507	3.538	-1.031	0.1393	10	10	10.47	72
Vaccine 7 vs. Control 5	2.507	3.485	-0.9777	0.1393	10	10	9.926	72
Vaccine 7 vs. Control 6	2.507	3.476	-0.9691	0.1393	10	10	9.838	72
Vaccine 8 vs. Control 1	2.548	3.718	-1.170	0.1393	10	10	11.88	72
Vaccine 8 vs. Control 2	2.548	3.538	-0.9900	0.1393	10	10	10.05	72
Vaccine 8 vs. Control 5	2.548	3.485	-0.9366	0.1393	10	10	9.508	72
Vaccine 8 vs. Control 6	2.548	3.476	-0.9279	0.1393	10	10	9.419	72
Control 1 vs. Control 2	3.718	3.538	0.1798	0.1393	10	10	1.826	72
Control 1 vs. Control 5	3.718	3.485	0.2333	0.1393	10	10	2.368	72
Control 1 vs. Control 6	3.718	3.476	0.2420	0.1393	10	10	2.456	72
Control 2 vs. Control 5	3.538	3.485	0.05346	0.1393	10	10	0.5427	72
Control 2 vs. Control 6	3.538	3.476	0.06213	0.1393	10	10	0.6307	72
Control 5 vs. Control 6	3.485	3.476	0.008674	0.1393	10	10	0.08805	72

Table A.5 Larval differentiations from coprocultures

Date	Plot	Haem	Trich	Tela	Oesoph	Other	Total
03-Jan	1	100	0	0	0	0	100
	2	98	2	0	0	0	100
	3	97	3	0	0	0	100
	4	100	0	0	0	0	34
	5	100	0	0	0	0	100
	6	100	0	0	0	0	37
	7	98	2	0	0	0	100
	8	100	0	0	0	0	100
16-Jan	1	100	0	0	0	0	100
	2	98	2	0	0	0	100
	3	79	21	0	0	0	100
	4	77	23	0	0	0	100
	5	98	2	0	0	0	100
	6	100	0	0	0	0	100
	7	100	0	0	0	0	100
	8	88	12	0	0	0	100
30-Jan	1	98	2	0	0	0	100
	2	90	10	0	0	0	100
	3	65	35	0	0	0	100
	4	81	19	0	0	0	100
	5	96	4	0	0	0	100
	6	100	0	0	0	0	100
	7	94	6	0	0	0	100
	8	91	9	0	0	0	100
13-Feb	1	98	2	0	0	0	100
	2	100	0	0	0	0	100
	3	70	22	0	0	8 Strongyloides	100
	4	84	16	0	0	0	100
	5	92	8	0	0	0	100
	6	100	0	0	0	0	100
	7	96	4	0	0	0	100
	8	96	10	0	0	0	100
27-Feb	1	96	4	0	0	0	100
	2	100	0	0	0	0	100
	3	45	26	0	0	29 Strongyloides	100
	4	65	35	0	0	0	100
	5	98	2	0	0	0	100
	6	100	0	0	0	0	100
	7	80	20	0	0	0	100

	8	93	7	0	0	0	100
13-Mar	1	98	2	0	0	0	100
	2	99	1	0	0	0	100
	3	32	68	0	0	0	100
	4	28	72	0	0	0	100
	5	81	2	0	0	17 Strongyloides	83
	6	100	0	0	0	0	100
	7	44	54	0	0	2 Strongyloides	98
	8	76	24	0	0	0	100
27-Mar	1	100	0	0	0	0	100
	2	97	3	0	0	0	100
	3	65	35	0	0	0	100
	4	64	34	0	2	0	100
	5	98	2	0	0	0	100
	6	96	0	0	0	4 Strongyloides	96
	7	80	18	0	0	2 Strongyloides	98
	8	96	4	0	0	0	100
11-Apr	1	100	0	0	0	0	100
	2	100	0	0	0	0	100
	3	52	38	10	0	0	100
	4	71	24	3	2	0	100
	5	98	1	1	0	0	100
	6	100	0	0	0	0	100
	7	90	9	1	0	0	100
	8	85	12	3	0	0	100
24-Apr	1	100	0	0	0	0	100
	2	100	0	0	0	0	100
	3	48	49	3	0	0	100
	4	81	18	1	0	0	100
	5	100	0	0	0	0	100
	6	100	0	0	0	0	100
	7	97	3	0	0	0	100
	8	76	23	1	0	0	100

Table A.6 Tracer lamb worm counts

Tracer Group 1 - on plots 13-27 February 2013

Number of worms in a 3% sample

				Haemonchus				Teladorsagia				Trichostrongylus			
Tag	Plot	Grp	Rep	Male	Female	Immature	Total	Male	Female	Immature	Total	Male	Female	Immature	Total
7004	2	C	1	14	11	0	25	44	44	0	88	0	0	0	0
7069	2	C	1	31	26	0	57	13	10	0	23	1	1	0	2
7043	5	C	2	19	18	0	37	0	3	0	3	0	0	0	0
7074	5	C	2	7	12	0	19	0	0	0	0	0	0	0	0
7028	6	C	3	3	6	0	9	1	2	0	3	0	0	0	0
7060	6	C	3	9	8	0	17	0	0	0	0	0	0	0	0
7048	1	C	4	19	22	0	41	0	2	0	2	0	0	0	0
7119	1	C	4	69	70	0	139	26	34	0	60	1	2	0	3
7131	3	V	1	11	8	0	19	1	6	0	7	0	0	0	0
7142	3	V	1	3	5	0	8	2	5	0	7	1	0	0	1
7063	4	V	2	3	5	0	8	0	1	0	1	0	1	0	1
7080	4	V	2	4	7	0	11	0	2	0	2	0	0	0	0
7125	7	V	3	10	7	0	17	2	2	0	4	0	0	0	0
7153	7	V	3	7	4	0	11	1	2	0	3	0	0	0	0
7108	8	V	4	11	6	0	17	2	2	0	4	0	0	0	0
7127	8	V	4	13	17	0	30	5	2	0	7	0	0	0	0

Plot means		Total number of *Haemonchus*						
		Male	Female	Immature	Total	Log total	V Feb	C Feb
1	1	750.00	616.67	0	1366.67	3.14	3.14	2.65
1	2	433.33	500.00	0	933.33	2.97	2.97	2.50
1	3	200.00	233.33	0	433.33	2.64	2.64	2.67
1	4	1466.67	1533.33	0	3000.00	3.48	3.48	2.89
2	1	233.33	216.67	0	450.00	2.65		
2	2	116.67	200.00	0	316.67	2.50		
2	3	283.33	183.33	0	466.67	2.67		
2	4	400.00	383.33	0	783.33	2.89		
Mean	**C**	**712.50**	**720.83**	**0.00**	**1433.33**			
Mean	**V**	**258.33**	**245.83**	**0.00**	**504.17**			

Tracer Group 2 - on plots 13-27 March 2013

Number of worms in a 3% sample

				Haemonchus				Teladorsagia				Trichostrongylus			
Tag	Plot	Grp	Rep	Male	Female	Immature	Total	Male	Female	Immature	Total	Male	Female	Immature	Total
7011	2	C	1	34	56	0	90	20	10	0	30	1	1	0	2
7214	2	C	1	48	51	0	99	4	14	0	18	1	1	0	2
7015	5	C	2	71	56	0	127	14	17	0	31	0	0	0	0

7136	5	C	2	22	18	0	40	2	6	0	8	1	0	0	1
7053	6	C	3	13	11	0	24	0	0	0	0	0	0	0	0
7082	6	C	3	16	12	0	28	0	0	0	0	0	0	0	0
7095	1	C	4	123	141	0	264	10	22	0	32	0	0	0	0
7143	1	C	4	35	45	0	80	11	17	0	28	0	0	0	0
7020	3	V	1	19	20	0	39	12	16	0	28	5	8	0	13
7041	3	V	1	13	17	0	30	5	13	0	18	3	4	0	7
7050	4	V	2	4	5	0	9	1	1	0	2	3	3	0	6
7152	4	V	2	2	3	0	5	1	1	0	2	0	0	0	0
7047	7	V	3	6	5	0	11	2	3	0	5	0	0	0	0
7098	7	V	3	4	7	0	11	6	7	0	13	0	0	0	0
7089	8	V	4	24	19	0	43	47	15	0	62	0	0	0	0
7112	8	V	4	25	25	0	50	13	32	0	45	0	0	0	0

Total number of *Haemonchus*

		Male	Female	Immature	Total	Log total	V Mar	C Mar
1	1	1366.67	1783.33	0	3150.00	3.50	3.50	3.06
1	2	1550.00	1233.33	0	2783.33	3.44	3.44	2.37
1	3	483.33	383.33	0	866.67	2.94	2.94	2.56
1	4	2633.33	3100.00	0	5733.33	3.76	3.76	3.19
2	1	533.33	616.67	0	1150.00	3.06		
2	2	100.00	133.33	0	233.33	2.37		
2	3	166.67	200.00	0	366.67	2.56		
2	4	816.67	733.33	0	1550.00	3.19		

Mean	**C**	**1508.33**	**1625.00**	**0.00**	**3133.33**
Mean	**V**	**404.17**	**420.83**	**0.00**	**825.00**

Tracer Group 3 - on plots 10-24 April 2013

Number of worms in a 3% sample

				Haemonchus				Teladorsagia				Trichostrongylus			
Tag	Plot	Grp	Rep	Male	Female	Immature	Total	Male	Female	Immature	Total	Male	Female	Immature	Total
7042	2	C	1	30	22	0	52	3	8	0	11	2	2	0	4
7123	2	C	1	30	33	0	63	0	0	0	0	0	3	0	3
7029	5	C	2	8	4	0	12	0	3	0	3	0	1	0	1
7040	5	C	2	12	19	0	31	5	6	0	11	3	1	0	4
7025	6	C	3	3	7	0	10	0	2	0	2	0	0	0	0
7122	6	C	3	0	6	0	6	0	1	0	1	0	0	0	0
7001	1	C	4	18	35	0	53	6	2	0	8	0	2	0	2
7030	1	C	4	19	23	0	42	0	3	0	3	0	1	0	1
7013	3	V	1	1	0	0	1	4	4	0	8	1	3	0	4
7016	3	V	1	3	6	0	9	8	9	0	17	1	0	0	1
7087	4	V	2	0	2	0	2	1	1	0	2	1	1	0	2
7154	4	V	2	1	1	0	2	0	2	0	2	0	0	0	0
7126	7	V	3	2	1	0	3	1	3	0	4	0	0	0	0
7137	7	V	3	1	5	0	6	2	1	0	3	0	0	0	0
7044	8	V	4	6	5	0	11	9	18	0	27	0	1	0	1
7058	8	V	4	3	3	0	6	5	7	0	12	0	0	0	0

		Total number of ***Haemonchus***						
		Male	Female	Immature	Total	Log total	V	C
1.00	1	1000.00	916.67	0	1916.67	3.28	3.28	2.22
1.00	2	333.33	383.33	0	716.67	2.86	2.86	1.82
1.00	3	50.00	216.67	0	266.67	2.43	2.43	2.18
1.00	4	616.67	966.67	0	1583.33	3.20	3.20	2.45
2.00	1	66.67	100.00	0	166.67	2.22		
2.00	2	16.67	50.00	0	66.67	1.82		
2.00	3	50.00	100.00	0	150.00	2.18		
2.00	4	150.00	133.33	0	283.33	2.45		
Mean	**C**	**500.00**	**620.83**	**0.00**	**1120.83**			
Mean	**V**	**70.83**	**95.83**	**0.00**	**166.67**			

Haemonchus worm counts obtained from the tracer lambs during February, March and April were divided by 14 to obtain the number of worms acquired per day for the graph in Fig 4b.

The output from analysis of variance of the tracer *Haemonchus* counts (log transformed) is shown below.

Number of families	1							
Number of comparisons per family	3							
Alpha	0.05							
Sidak's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary				
Vaccine Feb vs. Control Feb	0.3757	-0.2318 to 0.9833	No	ns				
Vaccine Mar vs. Control Mar	0.6140	0.006400 to 1.222	**Yes**	*				
Vaccine Apr vs. Control Apr	0.7723	0.1648 to 1.380	**Yes**	*				
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	t	DF
Vaccine Feb vs. Control Feb	3.055	2.679	0.3757	0.2309	4	4	1.627	18
Vaccine Mar vs. Control Mar	3.410	2.796	0.6140	0.2309	4	4	2.659	18
Vaccine Apr vs. Control Apr	2.941	2.169	0.7723	0.2309	4	4	3.345	18

Table A.7 Antibody titres

Plot	Vaccinates		Day 0	Day 28	Day 42	Day 57	Day 70	Day 84	Day 98	Day 112	Day 126	Day 140	Day 155	Day 168
3		21	3	43	7,692	6,142	10,257	6,921	6,949	12,270	8,923	7,910	13,601	16,206
		22	7	1,093	10,065	7,093	10,484	7,863	7,602	16,339	10,519	8,116	30,854	20,230
		23	347	1,203	6,035	4,852	8,195	6,121	5,813	13,228	8,846	7,556	12,554	10,164
		24	0	117	7,403	6,617	13,618	8,502	9,785	17,574	11,721	10,964	26,091	14,493
		25	11	580	10,656	9,969	106,854	32,812	16,264	110,404	42,249	20,978	172,100	36,680
		26	18	0	606	234	8,754	5,343	4,924	11,367	9,020	6,058	7,496	7,007
		27	5	279	9,131	7,482	18,148	9,648	8,325	17,692	11,348	8,987	20,450	12,813
		28	13	1,707	8,177	6,936	25,804	9,872	9,604	43,852	15,706	12,143	32,072	17,388
		29	0	1,782	6,644	6,160	8,290	5,887	5,639	9,025	7,100	6,421	7,858	6,747
		30	102	776	6,217	5,475	9,431	7,547	6,548	36,995	13,080	12,756	66,198	19,500
	mean		51	758	7,262	6,096	21,983	10,052	8,145	28,875	13,851	10,189	38,928	16,123
	sem		34	211	889	783	9,593	2,574	1,039	9,757	3,250	1,401	15,772	2,734

Plot	Vaccinates		Day 0	Day 28	Day 42	Day 57	Day 70	Day 84	Day 98	Day 112	Day 126	Day 140	Day 155	Day 168
4		31	30	0	6,121	5,606	9,512	6,917	7,095	10,411	8,463	8,096	12,394	10,937
		32	0	241	9,347	10,309	12,511	8,312	8,441	15,594	12,415	10,967	21,412	14,204

		33	14	1	6,276	5,653	11,083	7,251	6,810	14,982	10,883	8,713	12,924	9,812
		34	2,650	3,245	7,644	6,443	8,245	7,834	6,615	8,632	7,812	7,155	8,550	7,434
		35	37	13	5,752	4,825	4,686	4,856	4,008	8,498	7,122	6,368	9,019	8,141
		36	0	12	7,769	7,512	20,179	10,929	9,762	31,676	21,827	17,046	29,202	17,452
		37	7	26	8,499	7,106	11,078	7,718	7,299	11,416	8,899	7,847	11,552	9,366
		38	199	276	8,399	7,002	23,708	10,797	13,839	56,743	22,043	16,405	125,031	44,709
		39	16	1,339	7,620	6,602	27,323	8,736	8,513	16,476	10,025	8,194	11,067	8,280
		40	1	246	9,698	7,672	7,494	6,341	6,154	14,779	11,133	8,268	19,810	10,872
	mean		295	540	7,712	6,873	13,582	7,969	7,854	18,921	12,062	9,906	26,096	14,121
	sem		262	327	424	477	2,380	592	826	4,698	1,722	1,197	11,184	3,533
Plot	Vaccinates		Day 0	Day 28	Day 42	Day 57	Day 70	Day 84	Day 98	Day 112	Day 126	Day 140	Day 155	Day 168
7		61	1	1	8,575	7,713	17,082	9,540	9,014	14,697	10,940	8,871	17,643	12,849
		62	411	7	7,683	6,668	21,660	12,325	13,062	51,238	21,453	16,940	43,801	20,639
		63	111	43	7,432	5,928	8,269	6,902	6,214	10,061	7,543	7,538	9,210	7,151
		64	146	452	14,476	12,283	29,295	11,913	14,008	59,124	17,818	13,884	30,740	18,660
		65	3	23	7,930	6,344	9,471	6,111	5,855	9,585	7,671	6,369	9,922	7,464
		66	81	24	6,985	5,272	9,839	7,038	7,338	16,156	9,802	8,215	11,476	8,295

		67	17	84	10,652	8,481	42,209	11,925	9,791	51,935	17,435	13,713	21,562	11,923
		68	56	983	10,210	8,459	209,283	42,569	31,406	148,222	36,482	23,380	108,626	58,292
		69	0	5	7,848	7,196	30,519	14,107	11,657	54,344	22,525	18,150	49,463	25,936
		70	3	58	5,267	5,368	7,518	6,503	6,154	7,317	6,532	5,801	7,797	6,900
	mean		83	168	8,706	7,371	38,514	12,893	11,450	42,268	15,820	12,286	31,024	17,811
	sem		40	100	804	656	19,324	3,420	2,403	13,581	2,965	1,861	9,796	4,948
Plot	Vaccinates		Day 0	Day 28	Day 42	Day 57	Day 70	Day 84	Day 98	Day 112	Day 126	Day 140	Day 155	Day 168
8		71	66	12		8,192	14,596	9,399	8,838	12,575	9,038	8,023	10,686	8,884
		72	100	204	8,921	7,508	16,907	9,081	8,084	14,534	9,589	7,515	16,797	11,178
		73	57	169	8,820	6,815	12,880	8,171	7,255	13,542	10,569	9,042	17,202	12,278
		74	14	25	7,288	5,387	8,957	6,469	5,620	8,407	7,028	6,097	8,839	8,341
		75	0	828	9,711	9,757	17,534	9,000	7,811	11,938	8,673	7,550	14,286	9,743
		76	0	12	5,381	4,850	8,222	7,052	6,294	11,556	9,699	8,661	27,488	12,258
		77	0	0	6,940	6,221	33,761	12,698	10,517	18,998	13,611	10,171	18,675	12,232
		78	1	1,224	7,319	6,715	8,687	6,924	5,952	7,899	6,864	6,230	8,643	7,141
		79	60	1,063	8,312	7,230	13,824	8,317	7,086	21,220	13,174	10,227	25,142	13,053
		80	66	76	6,291	6,684	15,240	9,050	8,426	14,674	11,315	9,552	31,655	15,470

	mean		36	361	7,665	6,936	15,061	8,616	7,588	13,534	9,956	8,307	17,941	11,058
	sem		12	152	463	439	2,334	558	468	1,319	721	470	2,514	795

Plot	Controls		Day 0	Day 28	Day 42	Day 57	Day 70	Day 84	Day 98	Day 112	Day 126	Day 140	Day 155	Day 168
1		1	23					5						3,485
		2	2					612						966
		3	0					119						5,469
		4	0					0						2,105
		5	0					0						245
		6	22					240						1,650
		7	221					126						3,595
		8	23					9						4,007
		9	2					0						192
		10	0					9						896
	mean		27					112						2,261
	sem		11					61						567

Plot	Controls		Day 0	Day 28	Day 42	Day 57	Day 70	Day 84	Day 98	Day 112	Day 126	Day 140	Day 155	Day 168

2		11	0					26						3,868
		12	0					8						3,177
		13	22					48						422
		14	221					291						2,224
		15	25					2						979
		16	1					0						2,654
		17	0					1,761						1,377
		18	2,471					12						1
		19	5					109						1,116
		20	15					319						1,547
	mean		276					257						1,737
	sem		245					171						388

Plot	Controls		Day 0	Day 28	Day 42	Day 57	Day 70	Day 84	Day 98	Day 112	Day 126	Day 140	Day 155	Day 168
5		41	59					10						18
		42	5					26						893
		43	57					0						1,945
		44	3					27						1,386

		45	0					0						270
		46	0					0						1,146
		47	242					0						175
		48	76					196						1,868
		49	0					46						2,292
		50	1					0						19
	mean		44					31						1,001
	sem		24					19						272
Plot	Controls		Day 0	Day 28	Day 42	Day 57	Day 70	Day 84	Day 98	Day 112	Day 126	Day 140	Day 155	Day 168
6		51	30					28						712
		52	0					5						376
		53	60					304						1,940
		54	2					1						493
		55	5,647					297						1,431
		56	26					99						3,337
		57	0					2						1,499
		58	0					0						461

	59	2	511	3,671
	60	1,133	0	256
mean		690	125	1,418
sem		562	57	391

APPENDIX 4

WEATHER DATA
Data from Australian Bureau of Meteorology

Armidale airport- total monthly rainfall (mm)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
2012											*47.6*	*80.2*	
2013	*160.6*	*92*	*52.4*	*3.2*									

9.2 Appendix 2 – Report of replicated plot trial with lambs done by VHR

Veterinary Health Research Pty Ltd

Study Report

Study Title: A field study to test the efficacy of a *Haemonchus* vaccine in weaner sheep during times of high parasite challenge. New England district NSW, Australia.

Study No.:	**MIHO2860**		**Sponsor Study No.:**	N/A
Version No.:	**2**	**UNAUDITED DRAFT**	**Version Date:**	01 November 2013
Author:	S. Miller			

Sponsor:	Name: Address:	Julie Fitzpatrick Moredun Group Director Moredun Institute The Moredun Group Pentlands Science Park Bush Loan Penicuik Midlothian Scotland, UK
Sponsor Monitor and Representative:	Name: Address: Phone: E-mail:	David Smith The Moredun Group Pentlands Science Park Bush Loan Penicuik Midlothian Scotland, UK +44 (0)131 445 6131 David.Smith@moredun.ac.uk
Investigator:	Name: Quals.: Address:	Henry Chambers B. AgEc, Grad. Cert Rural Science Veterinary Health Research Pty Ltd Trevenna Road, Armidale, NSW 2350 Australia
Quality Assurance:	Name: Quals.: Address:	Leonora J. Pearson DipRQA Veterinary Health Research Pty Ltd Trevenna Road, Armidale, NSW 2350 Australia

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STUDY INVESTIGATOR

COMPLIANCE STATEMENT

I, the undersigned, hereby declare that the report is a complete, true and accurate representation of the study and its results.

This study was conducted in accordance with the approved Protocol and with VHR Standard Operating Procedures (see Appendix 2), unless otherwise stated, and the study objectives were achieved. The study was conducted in compliance with:

- VICH GL9 Good Clinical Practice (June 2000)

There were no deviations from Protocol or any other circumstances considered to have affected the outcome of the study

Signed: ______________________________

Henry Chambers BAgEc, Grad. Cert. RurSc
Study Investigator

Date: ______________________________

QUALITY ASSURANCE STATEMENT

Inspections were made by the Quality Assurance Unit of the various phases of the study described in this report. The date inspections were carried out and reported to the Investigator and to facility management are given below:

Inspection Date	Inspection Type	Phase Inspected	QA Auditor	Inspection Report issued

This report has been audited by the Quality Assurance Unit and is considered to be an accurate description of the methods and procedures used during the conduct of the study, and an accurate reflection of the raw data.

Signed: ______________________________

Leonora J Pearson DipRQA
Quality Assurance Manager

Date: ______________________________

1. Objective

To confirm the field efficacy of a *Haemonchus* vaccine in weaner sheep against *Haemonchus contortus*, when vaccinated and control sheep are grazed separately, in the New England region of New South Wales, Australia. Data from this study may be used to support product registration.

2. Justification

Traditionally the treatment of internal parasites in sheep has been via drenching with an anthelmintic compound to eradicate the parasites and with some compounds, kill the incoming larvae from the pasture. Parasite resistance to many of the commonly used anthelmintics is common in many parts of the world. The use of a vaccine to control these parasites would reduce dependence on anthelmintics, and hence be of great benefit to sheep producers, and for the welfare of the animal.

Initial field trials have shown that the vaccine in question is partially effective when control and vaccinated animals are grazed together. This study aimed to investigate the efficacy when conducted under 'label directions', where treated animals are grazed separately from untreated animals.

3. Compliance

The study was performed utilising internationally acceptable standards including VICH GL9 Good Clinical Practice such that the results may be presented for review by Moredun technical, regulatory and marketing personnel to government regulatory authorities.

4. Test sites

Animal Phase
Pre-weaning:
"Woodlands"
Torryburn Road
Uralla NSW 2358
Australia

Post-weaning:
"Waitahuna"
658 Cluny Rd
Armidale NSW 2350

Laboratory Phase:
Veterinary Health Research P/L
Colin Blumer Animal Health Laboratory
Trevenna Road
Armidale NSW 2350 Australia

Analytical Phase:
Moredun Institute
Pentlands Science Park
Bush Loan
Penicuik, Midlothian EH26 OPZ
Scotland, UK

5. Study dates

Start date (animal phase): 6 Nov 2012

Finish date (animal phase): 23 Apr 2013

Finish date (laboratory phase): 15 Aug 2013

6. Study design

The design was a randomised field study. Eighty (80) lambs on a commercial farm were identified using ear tags and allocated to two groups on the basis of weight and sex. Sheep in Group 1 (40 animals) were the control (unvaccinated) animals and had blood and faecal samples collected on the days shown in **Table 1**. Sheep in Group 2 (40 animals) were treated with the IVP and had blood and faecal samples collected on the days shown in **Table 1**.

The lambs remained with their mothers at the source property until weaning, whereupon the lambs were relocated to paddock facilities controlled by VHR. The plan was that the eighty (80) lambs were to be distributed equally across 8 paddocks, each of 10 sheep, but four lambs were missing on the day of weaning, two from each group. Thus two vaccinated and two control paddocks contained only 9 sheep each

An additional forty eight (48) randomly selected yearling sheep, sourced from the same property, were selected and allocated to a third group (Group 3) to be used as tracer animals to indicate at intervals the level of *H.contortus* challenge. The tracers were treated in three cohorts, each consisting of 16 animals, with a short-acting anthelmintic and maintained in undercover pens for two weeks to ensure 'clean-out' prior to introduction into each of the 8 trial paddocks (2 tracer animals per paddock, 16 in total per introduction). Fourteen days later these tracer animals were removed from the trial paddocks and placed in undercover pens again for a further two weeks before being killed for total worm counts at intervals outlined in Table 1.

Table 1 - Treatment Schedule

Group	Number of animals	Treatment	Treatment days	Kill for Total Worm Counts	Faecal sample collection days	Blood (plasma) sample collection for ELISA
1	40	Negative control	Day 21 (short-acting anthelmintic)	N/A	Days 0, 21, 42, 56, 70, 84, 98, 112, 126, 140, 154 and 168	Days 0, 84 and 168
2	40	IVP	Days 0, 21, 42, 84 and 126	N/A	Days 0, 21, 42, 56, 70, 84, 98, 112, 126, 140, 154 and 168	Days 0, 21, 42, 56, 70, 84, 98, 112, 126, 140, 154 and 168
3	48	Tracers	Days 63, 84, and 112	Days 98, 126 and 154	Day 0	N/A
Ewes	80	N/A	N/A	N/A	Day 0	

a. **Experimental Unit:** The experimental unit was the individual animal pre-weaning and the trial plots post weaning.

b. **Animal Model:** The study utilised young sheep (lambs born in spring 2012) for Groups 1 and 2 due to their susceptibility to nematode infection. Tracer animals were hoggets sourced from the same property as the Group 1 and 2 study animals. Study animals grazed paddocks contaminated by *Haemonchus contortus*, so that the infection was naturally acquired.

c. **Inclusion Criteria:** Animals were selected for the study if they met the criteria outlined in section 10 below.

d. **Exclusion and Removal Criteria:** No animals were excluded or removed from the study.

e. **Allocation:** 40 male and 40 female lambs were randomly selected from a flock, after excessively heavy or light ("outliers", up to ~10% of the flock) animals had been excluded. All selected trial animals were weighed on Day 0 and ranked from heaviest to lightest within each sex. The forty (40) animals within each sex were re-ranked by bodyweight, sequentially blocked into groups of two (2) animals and randomly allocated, utilising the "draw from hat" methodology from each of the two blocked groups to form two (2) treatment groups, each of 40 animals (Groups 1 and 2). Both groups had a similar mean and range of bodyweights with no statistical difference (P=0.05) between each group, and equal numbers of each sex.

At the time of relocation to facilities controlled by VHR (Day 63), Groups 1 and 2 animals (total 76 animals, see NTF1) were faecal sampled and ranked on Day 63 faecal strongyle egg counts from highest to lowest within each group, sequentially blocked into blocks of four (4) animals and randomly allocated, utilizing the "draw from hat" methodology to form two lots of four (4) groups, each of 9 or 10 animals. These 8 "grazing groups" were then randomly allocated via "draw from hat method" to similar discrete grazing paddocks such that each paddock contains 9 or 10 animals initially drawn from either the Group 1 (negative control) or Group 2 (IVP) animals.

The method of allocation and randomisation data sheets is included in the raw data - **APPENDIX 7.** Group mean bodyweights at allocation were analysed for significant differences between groups using One-Way Analysis of Variance and a commercially available software package (Statistix 9.0, 2008).

Forty-eight (48) Group 3 animals (tracers) were purchased from a commercial source and were randomly allocated to one of three cohorts as they appeared in the race on their respective "clean-out" days as per **Table 1 – Treatment Schedule**. (See Deviation #1).

f. Blinding: Laboratory personnel were blinded to treatment groups when performing faecal egg counts and total worm counts.

7. Investigational veterinary product

g. Investigational Veterinary Product:

Name:	BarberVax	Batch No.:	V1. HCD220311D-005 V2. HCD220311C-006 V3. HCD220311C V4. HCD220311D-005 V5. HCD220311D-007
Composition:	Haemonchus antigen and saponin adjuvant	Expiry Date:	20 June 2012 (V1,2,3,4) October , 2013(V5)
Dose Rate:	5ug antigen and 1mg saponin	WHP:	12 Months

h. Source: The IVP was provided in a ready to use form from the laboratory engaged by the Sponsor manufacture the vaccine. The laboratory was:

WormVax Laboratory
Animal Health Laboratory
Dept of Agriculture and food Western Australia
444 Albany Highway
Albany W.A 6330

i. Storage: The IVP was stored in a temperature monitored refrigerator on VHR premises between 2 and 8 oC. On treatment days it was transported to the trial sites in an esky on frozen ice packs.

j. Safety: A MSDS was not provided by the Sponsor (see Deviation 6).

k. Assays: A Certificate of Analysis was not provided for the IVP (see Deviation 6).

l. Drug Disposal: All remaining IVP was retained at VHR pending disposal advice from the Sponsor. Disposal of remaining IVP will be documented.

8. Treatment

Animals in Group 1 were retained untreated as negative controls but individual animals in either Group 1 or 2 were treated with a short acting anthelmintic if:

- ***H.contortus*:** the egg count rose above 10,000 epg or if the blood haemoglobin concentration measured below 6.5 g/100mL
- **Other genera:** (indicated by larval differentiation): the individual animal egg count rose above 1500 epg. For a flock treatment, the upper limit is a mean of 1000 epg.

a. Dose Calculation: Dose volume was 1mL IVP by subcutaneous injection for each Group 2 animal. Anthelmintic treatment for tracers and salvage drenches were calculated according to label directions based on individual animal bodyweights recorded at Day 0, 84, 126 and 168.

b. Dose Preparation: The IVP was gently shaken immediately prior to treatment. Anthelmintic products used for cleanout and salvage treatments were prepared as per label directions.

c. Method of Dose Administration: Study animals were dosed according to the treatment regime detailed in Table 1 below.

Table 2 – Treatment Regime

Grp.	IVP and CP Details	Dose Volume	Route	Treatment. Day(s)	No. Anim.
1	Negative controls	---	---	Day 21 (short acting anthelminitic), thence as required for salvage	40
2	IVP	1mL	Subcut.	Days 0, 21, 42, 84 and 126	40
3	An effective short-acting anthelmintic	As per label directions	Oral	Days 64, 84, and 112	48

Treatment administration was performed according to VHR SOP FLD-410. Treatment administration was recorded on the "Animal Treatment Record". The IVP was administered using a multi-dose injection gun, subcutaneously to the left hand side of the neck. Anthelmintics were administered orally using either plastic disposable syringes or a generic multi-dose handpiece. Study animals were observed immediately post-treatment and this was recorded in the "Clinical Observations Record".

9. Schedule of events

Table 3 – Schedule of events

Study Day	Date	Event
Pre-study	Pre-study	OBTAINED Animal Ethics Committee approval; RECEIVED IVP from supplier. LOCATED suitable mob of sheep on a commercial sheep farm with a suitable infection of *Haemonchus contortus.* CONFIRMED suitably infected sheep were grazing the selected plots allowing "seeding" of plots prior to weaner introductions.
Day 0	06 NOV 12	WEIGHED and tagged 80 merino lambs (selected from a larger flock of 300, after light and heavy 'outliers' had been removed) and allocated to two groups (Groups 1 and 2, Deviation #1). COLLECTED faecal samples and single heparinized blood samples from Group 1 and 2 animals (Deviation #2). TREATED animals in Group 2 with the IVP (V1). COLLECTED faecal samples from 10 randomly selected ewes to define current property status. CONDUCTED faecal strongyle egg counts and pooled larval differentiation on all samples. PROCESSED Group 1 and 2 blood samples and stored plasma frozen as 2 replicates.
Day 21	27 NOV 12	COLLECTED single heparinised blood samples from Group 2 animals. PERFORMED Hb analysis crushside on Group 2 animals only. COLLECTED faecal sample from all animals in Groups 1 and 2. Submitted faecal samples to lab for individual FEC and group bulk coproculture for larval differentiation. PROCESSED all Group 2 blood samples and stored plasma frozen as 2 replicates. TREATED animals in Group 2 with the IVP (V2). TREATED all animals in Groups 1 and 2 -(Group 3 animals had not been identified as yet, see Amendment #2) with a short-acting anthelmintic for scour worms and early Haemonchus
Day 42	18 DEC 12	COLLECTED single heparinised blood samples from all Group 1 and 2 animals. PERFORMED Hb analysis crushside. COLLECTED faecal samples from all Group 1 and 2 animals. Submitted faecal samples to laboratory for individual FEC and group bulk coproculture for larval differentiation. PROCESSED all Group 2 blood samples and stored plasma frozen as 2 replicates. TREATED animals in Group 2 with the IVP (V3).
Day 44	20 DEC 12	PURCHASED 48 hoggets (Group 3 tracers) from source property "Woodlands" and transported to VHR facilities (as per Amendment #2).
Day 63	08 JAN 13	COLLECTED single heparinised blood samples from all Group 1 and 2 animals. PERFORMED Hb analysis crushside. COLLECTED faecal samples from all animals in Groups 1 and 2. Submitted faecal samples to laboratory for individual FEC and group bulk coproculture for larval differentiation. PROCESSED all Group 2 blood samples and stored plasma frozen as 2 replicates. WEANED lambs and RELOCATED to VHR facilities (see Deviation #5).
Day 64	09 JAN 13	ALLOCATED weaners to paddocks using Day 63 FEC results. Group 1 animals split into 4 paddocks of 10 animals each, Group 2 animals split into 4 paddocks of 10 animals each. WEIGHED and TAGGED all Group 3 tracers. Randomly SELECTED sixteen (16) Group 3 tracers (cohort 1) and TREATED with an effective short-acting anthelmintic and PLACED in undercover pens to ensure 'clean-out'.

Day 70	15 JAN 13	COLLECTED single heparinised blood samples from all Group 1 and 2 animals. PERFORMED Hb analysis crushside. COLLECTED faecal samples from all Group 1 and 2 animals. Submitted faecal samples to laboratory for individual FEC and group bulk coproculture for larval differentiation. PROCESSED all Group 2 blood samples and stored plasma frozen as 2 replicates. INTRODUCED sixteen (16) Group 3 (cohort 1) tracer animals to paddocks with trial animals. Two tracers were randomly placed into each of the eight paddocks.
Day 84	29 JAN 13	COLLECTED single heparinised blood samples from all Group 1 and 2 animals. PERFORMED Hb analysis crushside. Salvage drenched one animal with Hb < 6.5g/dL. COLLECTED faecal samples from all animals in Groups 1 and 2. Submitted faecal samples to laboratory for individual FEC and group bulk coproculture for larval differentiation. PROCESSED all Group 2 blood samples and stored plasma frozen as 2 replicates. WEIGHED Group 1 and 2 animals+ on scales which were verified with test weights of a known mass. VACCINATED (V4) all group 2 animals. REMOVED Group 3 (cohort 1) tracers from paddocks and placed into undercover pens. Randomly SELECTED a further sixteen (16) Group 3 (cohort 2) tracers and TREATED with an effective short-acting anthelmintic and PLACED in undercover pens to ensure 'clean-out'.
Day 98	12 FEB 13	COLLECTED single heparinised blood sample from all Group 1 and 2 animals. PERFORMED Hb analysis crushside. Salvage drenched all animals with Hb < 6.5g/dL, and animals which had individual epg > 10,000 at previous FEC. COLLECTED faecal sample from all animals in Groups 1 and 2 animals. Submitted faecal samples to laboratory for individual FEC and group bulk coproculture for larval differentiation. PROCESSED all Group 2 blood samples and stored plasma frozen as 2 replicates. SACRIFICED cohort 1 Group 3 tracers at Highland Pet Foods, Guyra. COLLECTED entire abomasum from each individual and PERFORMED gut washing to obtain total contents. Submitted abomasum contents to laboratory for individual total worm counts. INTRODUCED sixteen (16) Group 3 (cohort 2) tracer animals to paddocks with trial animals. Two tracers were randomly placed into each of the eight paddocks.
Day 112	26 FEB 13	COLLECTED single heparinised blood samples from all Group 1 and 2 animals. PERFORMED Hb analysis crushside. Salvage drenched all animals with Hb < 6.5g/dL. COLLECTED faecal samples from all animals in Groups 1 and 2. Submitted faecal samples to laboratory for individual FEC and group bulk coproculture for larval differentiation. PROCESSED all Group 2 blood samples and stored plasma frozen as 2 replicates. REMOVED Group 3 (cohort 2) tracers from paddocks and placed into undercover pens. Randomly SELECTED a further sixteen (16) Group 3 (cohort 3) tracers and TREATED with an effective short-acting anthelmintic and PLACED in undercover pens to ensure 'clean-out'.

Day 126	12 MAR 13	COLLECTED single heparinised blood samples from all Group 1 and 2 animals. PERFORMED Hb analysis crushside. Salvage drenched all animals with Hb < 6.5g/dL. COLLECTED faecal samples from all Group 1 and 2 animals. Submitted faecal samples to laboratory for individual FEC and group bulk coproculture for larval differentiation. PROCESSED all Group 2 blood samples and stored plasma frozen as 2 replicates. Drenched animals in paddock G1 (Group 2) for Trichostrongylus and Ostertagia spp (see NTF 4)WEIGHED Group 1 and 2 animals on scales which were verified with test weights of a known mass. VACCINATED (V5) all group 2 animals. SACRIFICED cohort 2 Group 3 tracers at Highland Pet Foods, Guyra. COLLECTED entire abomasum from each individual and PERFORMED gut washing to obtain total contents. Submitted abomasum contents to laboratory for individual total worm counts. INTRODUCED sixteen (16) Group 3 (cohort 3) tracer animals to paddocks with trial animals. Two tracers were randomly placed into each of the eight paddocks.
Day 140	26 MAR 13	COLLECTED single heparinised blood samples from all Group 1 and 2 animals. Performed Hb analysis crushside. Salvage drenched all animals with Hb < 6.5g/dL. COLLECTED faecal samples from all Group 1 and 2 animals. Submitted faecal samples to laboratory for individual FEC and group bulk coproculture for larval differentiation. PROCESSED all Group 2 blood samples and stored plasma frozen as 2 replicates. REMOVED Group 3 (cohort 3) tracers from paddocks and placed into undercover pens.
Day 154	09 APR 13	COLLECTED single heparinised blood sample from all Group 1 and 2 animals. PERFORMED Hb analysis crushside. Salvage drenched all animals with Hb < 6.5g/dL. COLLECTED faecal sample from all animals in Groups 1 and 2 animals. Submitted faecal samples to laboratory for individual FEC and group bulk coproculture for larval differentiation. PROCESSED all Group 2 blood samples and stored plasma frozen as 2 replicates. SACRIFICED cohort 3 Group 3 tracers at Highland Pet Foods, Guyra. COLLECTED entire abomasum from each individual and PERFORMED gut washing to obtain total contents. Submitted abomasum contents to laboratory for individual total worm counts.
Day 168	23 APR 13	COLLECTED single heparinised blood samples from all Group 1 and 2 animals. PERFORMED Hb analysis crushside. COLLECTED faecal samples from all animals in Groups 1 and 2. Submitted faecal samples to laboratory for individual FEC and group bulk coproculture for larval differentiation. PROCESSED all Group 1 and 2 blood samples and stored plasma frozen as 2 replicates. WEIGHED Group 1 and 2 animals on scales which were verified with test weights of a known mass. DRENCHED all animals in groups 1 and 2 with an effective anthelmintic to individual bodyweights. COMPLETION OF ANIMAL PHASE.
Day 168 +	Day 168 +	DISPATCHED replicate 1 samples to Moredun Institute, Scotland. COLLATED data, compile report.

10. Test system

Species:	Ovine	**Number:**	128
Breed:	Merino	**Source:**	"Woodlands" Torryburn Road, Uralla, NSW 2358
Weight:	9 - 25 kg (Groups 1 and 2) 29 – 54.5 kg (Group 3)	**Health and special requirements:**	Healthy animals. Not within existing WHP and ESI for animal health products used.
Sex:	Equal numbers of male castrates and females		
Age:	6 weeks – groups 1 and 2 14 months – group 3	**Method of ID:**	Individually numbered eartags, coloured group eartags.

11. Animal management

a. Animal Welfare: Study animals were managed similarly and with due regard for their welfare. Animals were observed twice weekly for health problems according to AEC requirements. Animals were handled in compliance with UNE AEC no. AEC12-103 and any applicable local regulations.

b. Health Management: All lambs in Groups 1 and 2 were vaccinated by the grazier with 5-in-1 for clostridial diseases prior to study commencement.

Any health problems or adverse events occurring during the study were recorded:

Day 126 (12 Mar 13): Animal #59 (Group 2) and #46 (Group 2) were both suffering from flystrike. Both animals were clipped around the affected area and treated with PROGUARD. Additionally animal #46 was also treated with 4ml ENGEMYCIN 100.

Day 140 (26 Mar 13): Animal #69 (Group 1) and #80 (Group 1) were both suffering from flystrike. Both animals were clipped around the affected area and treated with EXTINOSAD - AEROSOL FOR WOUNDS.
Animal #46 (Group 2) was still displaying signs of severe flystrike, so was also treated with EXTINOSAD - AEROSOL FOR WOUNDS.

c. Deaths: Three animals were found dead in their paddocks during the plot phase of the study. Two animals (#40 and #23, Group 1) died between the Day 140 and Day 154 sampling points. #40 was salvage drenched on Day 140. The intervention was triggered by a low Hb value. #23 had been last drenched on Day 112 and had a FEC of 0 on Day 140. #74 (Group 2) died between the Day 112 and Day 126 sampling points. Cause of death was not established however the deaths occurred during a time of extreme blowfly challenge.

d. Housing: Routine management practices were followed. All Group 1 and 2 trial animals were maintained as one grazing mob with their mothers until weaning on Day 63. The trial animals were then relocated to VHR facilities and maintained as one group overnight until they were then allocated into 8 groups of 10 animals on Day 64. Each group had *ad-lib* access to a range of native and improved pastures. Potable water was also supplied *ad-lib*.

Tracer animals (group 3) were purchased and relocated to VHR facilities on Day 44 where they were maintained as one grazing mob with *ad-lib* access to native and improved pastures. Tracer animals were also housed in undercover worm-free pens at intervals outlined in Section 9 – Schedule of Events, at which animals were fed a combination of oaten and Lucerne chaff through plastic bins daily at a rate formulated to maintain normal growth patterns.

Tracer animals also grazed with group 1 and 2 animals at intervals outlined in Section 9 – Schedule of Events.

All animals had *ad-lib* access to potable water through plastic troughs at all times.

e. Animal Disposal: Study animals in Group 3 were euthanased according to VHR SOP FLD-430 at intervals outlined in Section 9 - Schedule of Events. Group 1 and 2 animals were retained on VHR property at the conclusion of the study where animals in Group 2 will not enter the human food chain for 12 months from the last treatment with the IVP on Day 126. Animals in Group 1 may be sold once they are out of the withhold period and export slaughter interval for the effective anthelmintic treatment administered on Day 168. An "Animal Accountability" form will be completed.

12. Study procedures

a. Trial Log: All scheduled and unscheduled events during the study were recorded

b. Informed Consent: An "Owner Consent and Agreement" form was signed by the Owner and the Investigator prior to administration of treatment.

c. Weather Data: Data from the nearest Bureau of Meteorology weather station for the study period are included in the raw data.

d. Sample Storage, Transfer and Disposal: Sample storage, transfer and disposal were recorded. Replicate 1 plasma samples were dispatched to Moredun Institute for analysis via WORLD COURIER on 24 June 2013. Replicate 2 plasma samples were held in frozen storage at VHR facilities for a period of 12 months after the last sample collection timepoint, after which point they will be disposed of by high temperature incineration.

13. Assessment of effects

a. **Body Weights:** Animals were weighed at intervals outlined in section 9 - Schedule of Events and individual animal weights were recorded. Animal weigh scales were checked pre- and post-weighing with calibrated test weights. Body weights and body weight change during the study were compared between groups to determine treatment effects, if any, and are detailed in the results section of the Study Report.

b. **Blood Analysis:** Single blood samples were collected using 18 gauge needles into 8 mL lithium heparin gel separated vacutainers, in accordance with VHR SOP FLD-414, at intervals outlined in section 9 – Schedule of Events. Blood samples were processed for collection of plasma samples on the day of collection, or following overnight refrigeration. Samples were individually labeled with the study no., animal no., study date and day, sample type. Frozen plasma samples were forwarded to Moredun Institute for ELISA antibody analysis. Results were compared to determine treatment effects, if any, are detailed in the results section of the Study Report.

c. **Faecal Egg Counts / Larval Differentiation:** Faecal samples were collected at intervals outlined in section 9 – Schedule of Events. Faecal samples were individually labeled with the animal ID. Individual faecal egg counts and bulk/group larval differentiation were performed. Faecal egg counts and larval differentiation were compared to determine treatment effects, if any (see **Tables 6 and 7, Appendix 3**), and are detailed in the results section of the Study Report.

d. **Worm Counts:** All animals in Group 3 were humanely euthanased at intervals outlined in Section 9 – Schedule of Events, and abomasums collected, according to VHR SOP 1. Total worm counts were performed and recorded (see **Table 9, Appendix 3**). Total worm counts were compared to determine treatment effects, if any, and are detailed in the results section of the Study Report.

e. **Haemoglobin:** Single blood samples were collected using 18 gauge needled into 8mL lithium heparin gel sepearted vacutainers in accordance with VHR SOP FLD-414, at interval outlined in section 9 – Schedule of Events. A sub-sample was used to determine haemoglobin content using a Hemocue 201Hb Analyser. The subsequent result was used determine if a salvage treatment was necessary. Haemoglobin data are presented in **Table 8, Appendix 3.**

14. Statistical analysis

This was done using the "GraphPad Prism" program. See output of statistical tests in **Appendix 4.**

15. Quality assurance

Veterinary Health Research has an independent Quality Assurance Unit which reviewed all aspects of quality assurance relating to this study. The Protocol, Study Report and raw data were subject to quality assurance inspection.

16. Data records

a. Amendments and Deviations:

Amendment #1: The Day 28 activity was removed from the trial design. Group 3 tracer animals were not placed on worm free paddocks as they were drenched with an effective anthelmintic to ensure clean-out and placed in worm free pens for 2 weeks *prior* to being introduced to group paddocks. This allowed maturation of and total clean-out of existing worm burden before group 3 animals were placed in group paddocks to act as tracers. This had a positive impact on the trial conduct due to technical rigour.

Amendment #2: Tracer animals were drenched and placed in worm free pens on Day 56. Lambs in groups 1 and 2 were weaned on Day 70, hence lambs of the same age and source could not be allocated to Group 3, tracers, as they would too young at Day 56. Group 3 animals were therefore hoggets, selected from a separate mob on the same farm, sometime prior to Day 56. This had a positive impact on the trial conduct due to technical rigour.

Deviation #1: This relates to Amendment #1. Allocation did not include lambs being allocated to Group 3, Tracers. Group 3 animals were hoggets, sourced separately. This had nil impact on the study.

Deviation #2: This relates to Deviation #1. Due to tracer animals being sourced after Day 0, no faecal sample was collected for Group 3 animals. This had nil impact on the study.

Deviation #3: Faecal and blood samples were not collected from 3 animals on Day 70, #21 (Group 1), #62 (Group 1) and #78 (Group 1). These animals had escaped from their group paddocks and did not present on the day. They were subsequently found and returned to rightful paddocks several days later. This had nil impact on the study.

Deviation #4: Animals #42 and #20 were treated with and anthelmintic for high *Trichostrongylus* and *Oestetargia* numbers, according to Day 154 FEC results. However, the results for the individual animal egg counts were lower than the required criteria of above 1,500 epg. This was due to error in calculations. This had nil impact on the study.

Deviation #5: Day 56 activities, along with weaning and the relocation of trial animals to VHR facilities were performed on Day 63 because Day 56 fell on New Years Day. This had nil impact on the study.

Deviation #6: No MSDS or Certificate of Analysis was provided by the Sponsor.

Deviation #7: Animals from paddock G1 (Group 2) were treated with a short acting anthelmintic on Day 126 due to high counts of Trichostrongylus and Ostertagia species from Day 112 bulk cultures. This was due to a large proportion of these species in the larval differentiation (19% and 31% respectively). The mean FEC of the group in this plot on D112 was 1896 EPG, with 48% (910 epg) of Haemonchus. The combined total of other spp. (as determined by the group coproculture) was 985 epg, 15 epg below the threshold outlined in Section 8 of the Protocol. Because the combined total was close to the threshold outlined in Section 8 of the Protocol it was decided (after discussion with the VHR Production Veterinarian) to drench with Ivermectin, the drench which would have the least effect on the Haemonchus burden of the group whilst still reducing

Trichostrongylus and Ostetargia. Treatment was done at the next sampling time point (D126).

Deviation #8: Trial animals were unnecessarily weighed on Day 126.

b. Notes to File:

Note to File 1: During weaning and subsequent transport of lambs to VHR facilities on Day 63, four lambs could not be found, and were therefore excluded from the trial: #47 and #58 (Group 2) and #71 and #79 (Group 1).

Note to File 2: Animal #23 (Group 1) and #40 (Group 1) died following the Day 140 sampling and #74 (Group 2) died after the Day 112 sampling. Therefore no samples were collected from these animals thereafter.

Note to File 3: An inadequate amount of faecal samples at Day 154 sample collection resulted in a nil or limited larval differentiation to be performed on those animals in paddocks G1, (Group 2), G2,G3 and G4 (Group 1).

Note to File 4: Group 3 tracers were moved into pens by cohort (after having a cleanout drench administered) prior to being put into the plots with the trial sheep. This was done to ensure cleanout and prevent re-infection before being used as tracers.

c. Change of Study Personnel :

Change of Study Personnel 1: Scott Miller replaced Jess McLeod as Investigator on 21 Jan 2013 due to Jess's departure from Veterinary Health Research.

Change of Study Personnel 2: Henry Chambers replaced Scott Miller as Investigator on 9 August 2013 due to Scott's departure from Veterinary Health Research.

d. Raw Data: All original raw data pages have been identified with the study number, signed and dated by the person making the observation and by the person recording the information, and will be paginated prior to appending to the final Study Report.

e. Communication Log: The Investigator maintained copies of all correspondence relating to the study. These will be archived with the final Study Report.

f. Permits: The study was covered by APVMA small trial permit no. PER 7250.

g. Confidentiality: Confidentiality of the raw data, Study Report and results of the study, plus any information received from the Sponsor, will be maintained during and after the study. Publication of material will remain at the sole discretion of the Sponsor.

h. Study Report: The original signed Study Report with raw data and Statistical Report appended will be submitted to the Sponsor. A copy of the Study Report, plus appendices, will be archived at Veterinary Health Research Pty Ltd, Trevenna Road, Armidale, NSW, Australia for a minimum of five years.

17. Results

a. Deaths and Data handling

Where more than 3 of the samples collected over the course of the trial were missing from a sheep, all the data from that animal was discarded from the analysis. This happened with 2 controls and 3 vaccinates

Haemonchus Egg Counts

When averaged over the trial, mean *Haemonchus* faecal egg counts of the lambs in the vaccinated plots were significantly ($p<0.01$) lower than those grazing the control plots (Fig 1a).

Examination of the overall trial average counts of each lamb (Fig 1b) identified only one "non-responder", defined as a vaccinate with a mean egg count greater than the lower 95% confidence limit of the control group. Two control lambs in Plot 4 had very low counts throughout the trial.

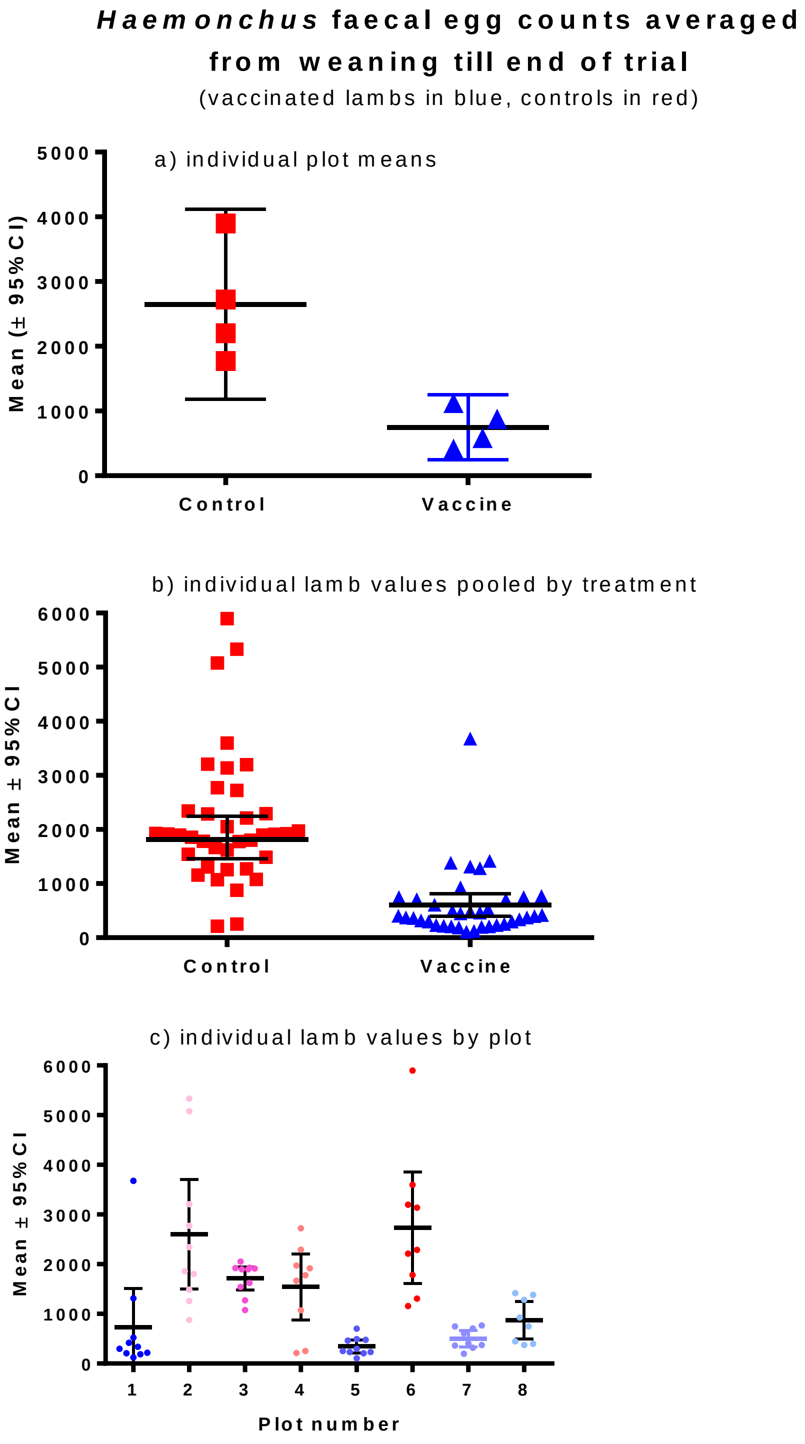


Fig 1. Mean *Haemonchus* egg counts from weaning to end of trial.

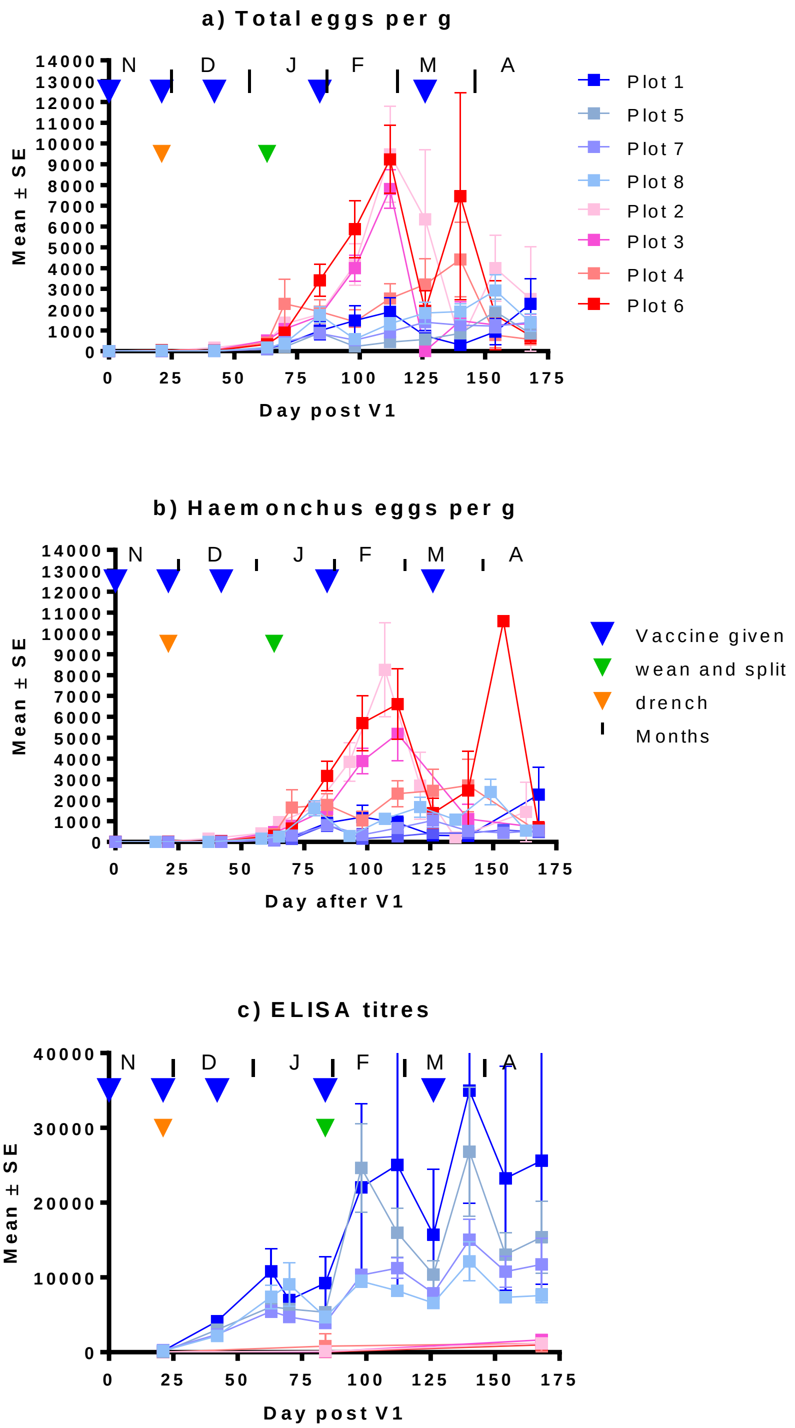


Fig 2. Kinetics of total and *Haemonchus* specific egg counts and the anti-vaccine antibody response in each plot. Note: control plots in red, vaccinated plots in blue.

Antibody concentrations did increase in the control lambs over the course of the trial, although nowhere near the scale of the vaccinates.

The first dose of vaccine did not stimulate a detectable serum antibody response. However, by the end of January, after the second and third immunisations had been administered, the mean titres of each vaccine plot ranged between 5 and 10,000 approximately (Fig 2c). Individual titres after the final pair of boosts were highly variable, but group means remained above 8,000 on all four plots.

Late January also saw the start of a marked increase in *Haemonchus* egg output on 3 of the control plots (Fig 2b or 3a). This peaked in late February attaining means of between 5 and 8,000 eggs per g, before dropping off after precautionary drenching (Fig 3c). On the remaining plot (no 4), the rise in egg count was more gradual and did not peak until late March.

In contrast, *Haemonchus* egg output on the vaccinated paddocks was significantly lower (Fig 2b), presumably due to the high titres of circulating antibody.

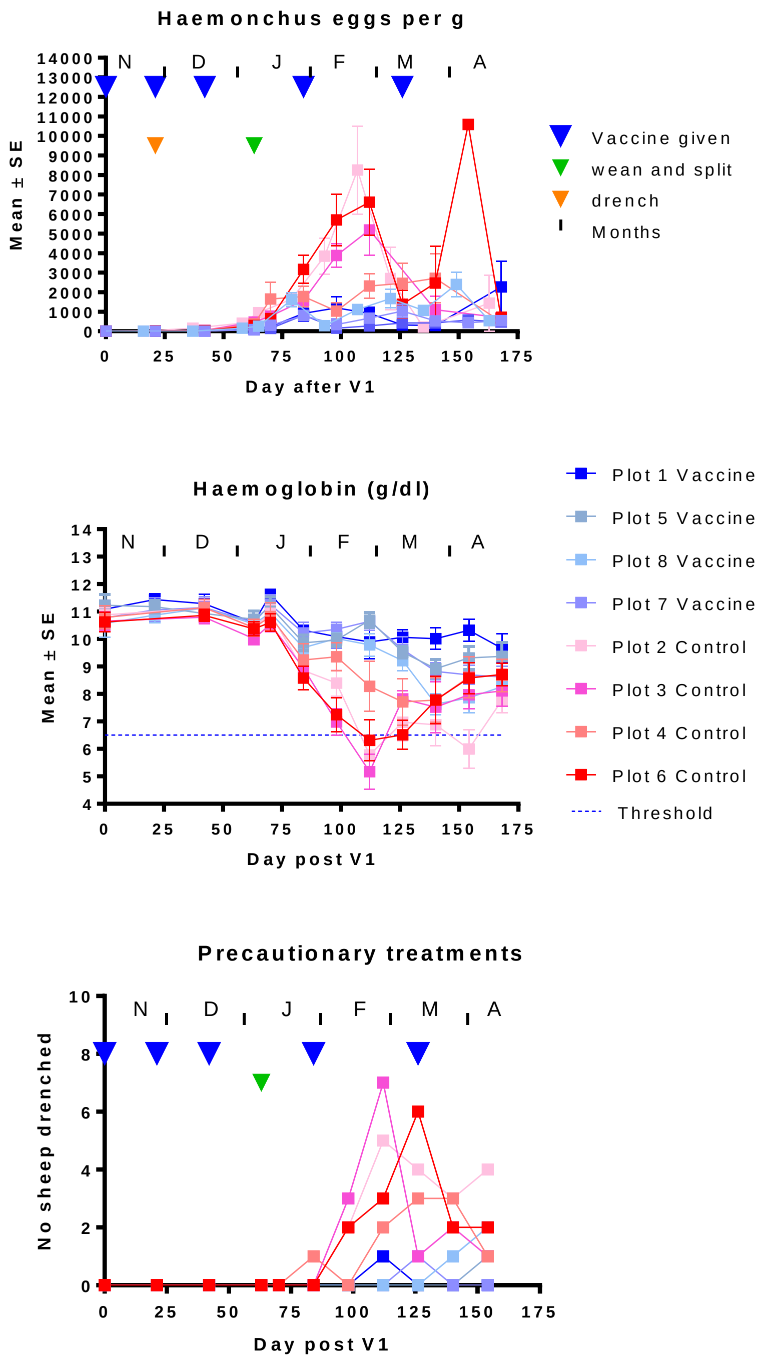


Fig 3. Kinetics of *Haemonchus* specific egg counts in relation to blood haemoglobin concentrations and the number of precautionary drenches given in each plot.

Note: control plots in red, vaccinated plots in blue.

The rise in *Haemonchus* egg counts in the control plots between mid January and mid February was associated with a corresponding drop in blood haemoglobin concentrations.

It was decided at the outset that any sheep with a haemoglobin level below 6.5g/dl (equivalent to a haematocrit of 22%) or with a faecal egg count above 10,000 eggs per g, would be given a precautionary drench. Only 2 of the 38 control sheep did not require such a drench throughout the trial, whereas the corresponding figure for the vaccinated lambs was 30 out of 38, a highly significant difference (p<0.001). Many sheep, especially the controls required more than one drench during the trial. Further details are presented in Table 1 below.

Table 4. Number of precautionary drenches given per plot and statistical significance of the data when pooled by treatment group.

Day after V1	**Plot no**								**Totals**		**Fishers exact**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**control**	**vaccinates**	**Test**
	v	c	c	c	v	c	v	v	N=38	N=38	P value
70	0	0	0	0	0	0	0	0	1	0	NS
84	0	0	0	1	0	0	0	0	7	0	0.02
98	0	2	3	0	0	2	0	0	17	0	0.001
112	1	5	7	2	0	3	0	0	14	1	0.001
126	0	4	1	3	0	6	1	0	10	1	0.01
140	0	3	2	3	0	2	0	1	8	1	0.05
154	0	4	1	1	1	2	0	2	4	3	NS
168	1	1	2	1	1	0	1	1	0	4	NS
Total									**61**	**10**	0.001

V denotes vaccinates, C denotes control

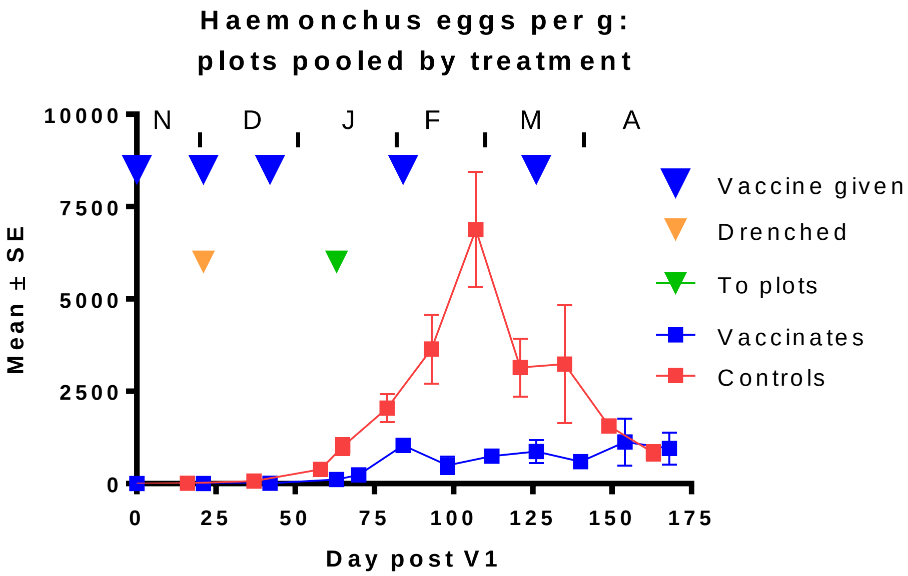
In order to measure the level of pasture contamination with *Haemonchus* larvae, two tracer hoggets grazed each plot for two weeks during January, February and March. The number of worms recovered from the abomasum of each animal is shown in Appendix 3 Table 9 and the mean number of *Haemonchus* acquired per day across all the vaccinated and control plots is presented in Fig 4.

The great majority of the worms recovered were *Haemonchus*, the remainder were predominantly *Teladorsagia* although a few *Trichostrongylus* were also found in some sheep.

Considerable individual variation in *Haemonchus* numbers was observed; the difference between a pair of tracers simultaneously grazing the same paddock was sometimes more than 10 fold (**Table 9, Appendix 3**). The fact that the tracers were hoggets may have contributed to the size of this variation, because having had a season's prior exposure to *Haemonchus*, some animals may have acquired a much stronger degree of natural immunity than others.

Whatever the reason, it can be seen in Fig 4b that the number of *Haemonchus* acquired on the control plots increased almost 6-fold between January and March. A similar rate of increase was observed on the vaccine plots between January and February but in March the number of worms acquired fell away to almost 7-fold below the equivalent control value. However, due to the large individual variation, this difference was not quite statistically different (p=0.053).

Sufficient rain fell between January and March for worm eggs to hatch into infective larvae (Fig 4b). If a 2-3 week delay is allowed for this to happen, then the change in pasture infectivity (i.e. the numbers of worm acquired by the tracers) seemed to reflect the size and kinetics of the mean *Haemonchus* egg output on the control and vaccinated plots quite well (**Figs 4a and 4b**).



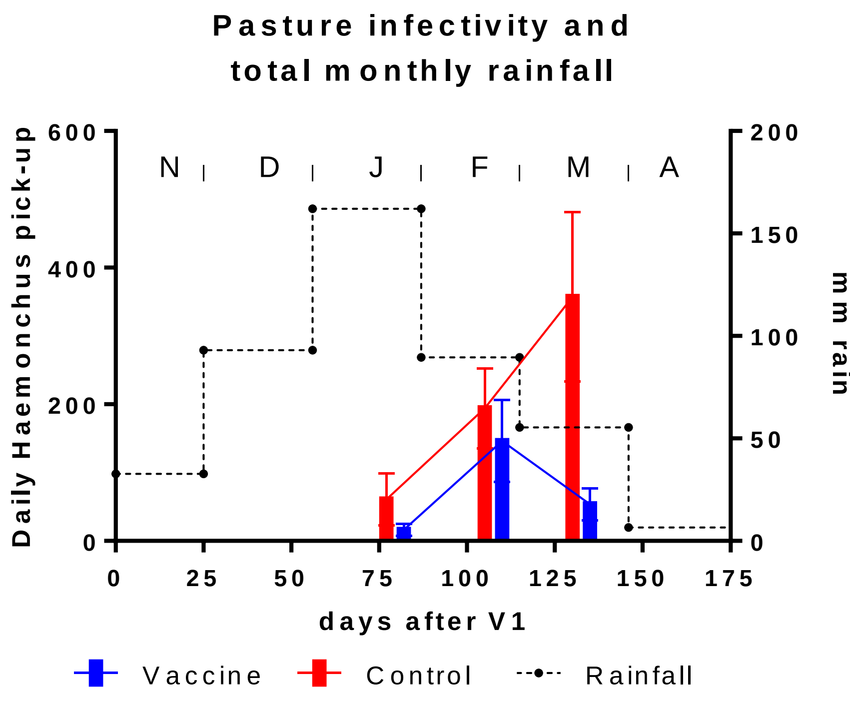


Fig 4. Kinetics of the infectivity of vaccine and control plots relative to the number of eggs being deposited on the pasture and the monthly rainfall.

c) Bodyweights.

These were measured on days 0, 84 and 168 when the trial finished. No significant differences were detected between the weights of the vaccinated and control groups at any stage of the trial

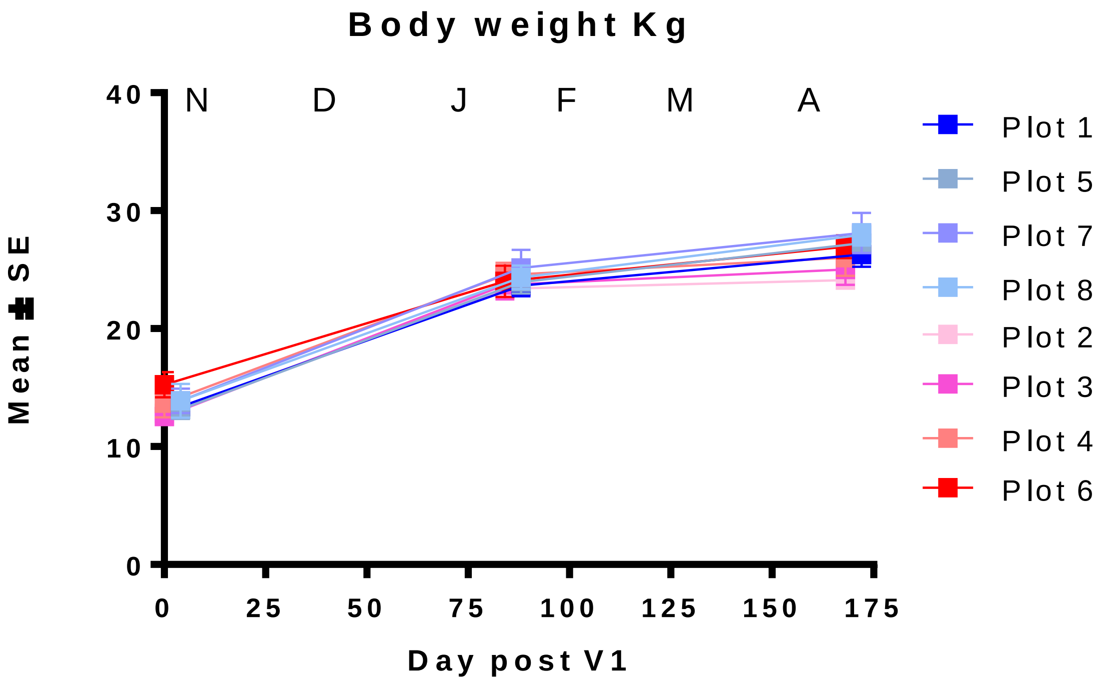


Fig. 5 Plot Mean Bodyweight over duration of trial.

18. Discussion

Vaccination stimulated significant serum antibody responses from after the second injection. As observed in previous trials each subsequent boost provoked a fairly short lived spike in titre with group mean titres increasing gradually as the trial progressed.

It was very clear that immunisation greatly depressed the *Haemonchus* egg counts of the lambs in the vaccine plots relative to those in the controls. This reduction in egg count presumably reflected a corresponding reduction in the number of blood feeding worms because the vaccinates were significantly less anaemic than the controls during February and March, when the control infestation was at its peak. As would be expected, reduced egg output on the vaccinate plots translated into a substantial reduction in pasture larval contamination by the end of the summer, providing an obvious epidemiological benefit to the immunised animals.

The protective effects portrayed in the figures above are obviously an underestimate of the true situation because almost all the control lambs received anthelmintic support, without which some would probably have succumbed to Haemonchosis.

APPENDIX 1

ABBREVIATIONS

VICH	International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Products
GCP	Good Clinical Practice
APVMA	Australian Pesticides and Veterinary Medicines Authority
MORAG	Veterinary Manual of Requirements and Guidelines
WAAVP	World Association for the Advancement of Veterinary Parasitology
VHR	Veterinary Health Research
SOP	Standard Operating Procedure
WHP	Withholding Period
ESI	Export Slaughter Interval
MSDS	Material Safety Data Sheet
ID	Identification
UNE	University of New England
AEC	Animal Ethics Committee
QA	Quality Assurance
kg	Kilograms
g	Grams
µg	Micrograms
mg	Milligrams
mL	Millilitres
Hb	Haemoglobin
Hc	*Haemonchus contortus*
Epg	Eggs per gram
AWI	Australian Wool Inovation

APPENDIX 2

STANDARD OPERATING PROCEDURES

SOP No.	SOP Title
STU-308	Test and Reference Item Receipt, Storage, Usage and Disposal
STU-309	Study File Set-up, Raw Data Collection, Error Correction and Storage
STU-311	Sample Identification, Storage, Transfer and Disposal
FLD-403	Identification of Study Animals
FLD-404	Feeding and Care of Study Animals
FLD-406	Weigh Study Animals and Weigh Scale Verification
FLD-408	Handling and Restraint of Study Animals
FLD-409	Health Monitoring in Study Animals
FLD-410	Chemical Application
FLD-414	Blood Sample Collection
FLD-416	Faecal Sample Collection
FLD-430	Euthanasia
PRO-506	Processing of Blood Samples for Assay Purposes
PRO-507	Gastrointestinal Nematode Recovery and Counting in Ruminants
PRO-510	Faecal Nematode Egg Counting using the Modified McMaster Method
PRO-512	Faecal Culture for the Recovery of Nematode Larvae

APPENDIX 3

TABULATED DATA

Table 5. Individual Bodyweight for Duration of Trial

			Bodyweight (kg)			
Paddock	**ID**	**Group**	**Day 0**	**Day 84**	**Day 126**	**Day 168**
G1	3	2	11.5	26.0	28.0	25.5
G1	17	2	13.5	22.5	24.0	25.0
G1	24	2	16.5	27.0	27.5	28.0
G1	39	2	16.0	27.5	30.5	31.0
G1	48	2	13.5	20.0	20.5	21.5
G1	56	2	13.5	24.5	27.0	29.5
G1	59	2	11.0	18.5	17.0	20.5
G1	66	2	11.0	23.0	25.0	26.0
G1	70	2	12.5	23.5	27.0	28.0
G1	73	2	15.0	24.0	28.5	28.0
G2	12	1	12.5	25.5	22.5	26.0
G2	15	1	17.5	27.5	24.0	25.5
G2	18	1	11.5	19.5	21.5	22.5
G2	21	1	12.5	21.0	21.0	22.0
G2	23	1	13.5	24.0	26.5	---
G2	32	1	11.5	22.5	23.5	23.5
G2	33	1	9.5	21.5	25.0	24.5
G2	38	1	12.0	22.0	20.5	24.0
G2	41	1	16.0	27.0	28.5	23.0
G2	80	1	11.5	23.5	25.5	26.0
G3	2	1	10.5	17.5	18.0	20.0
G3	7	1	14.5	26.0	26.5	26.0
G3	11	1	12.5	21.5	23.0	22.5
G3	29	1	13.0	23.5	24.5	26.0
G3	31	1	10.0	19.0	26.0	19.5
G3	40	1	12.0	25.5	32.0	---
G3	50	1	15.0	28.0	28.5	30.5
G3	55	1	15.0	28.5	30.0	29.5
G3	60	1	12.0	27.5	32.5	28.0
G3	78	1	11.0	20.0	26.0	23.0
G4	6	1	10.5	21.5	22.5	22.0
G4	35	1	13.0	24.0	18.5	24.0
G4	36	1	17.0	30.0	31.0	36.0
G4	37	1	16.5	26.5	32.5	26.5
G4	43	1	10.0	22.0	24.0	25.0
G4	44	1	11.5	24.0	29.5	23.5
G4	45	1	10.0	20.5	18.5	20.5

G4	49	1	16.0	28.0	25.0	29.5
G4	62	1	11.0	24.5	25.5	27.0
G4	79	1	21.5	---	---	---
G5	5	2	12.0	26.0	19.0	27.0
G5	8	2	17.5	26.5	21.0	29.5
G5	13	2	15.0	25.5	22.5	27.5
G5	14	2	10.0	24.0	17.5	26.5
G5	19	2	12.0	23.0	21.5	28.0
G5	22	2	11.0	20.5	13.0	25.0
G5	27	2	13.0	19.5	20.0	23.0
G5	53	2	13.5	23.5	22.5	27.0
G5	67	2	11.0	21.5	18.5	26.0
G5	76	2	16.5	29.5	26.0	33.0
G6	1	1	17.0	28.5	30.0	29.5
G6	28	1	13.0	23.0	24.5	26.5
G6	34	1	15.5	30.0	30.0	31.5
G6	54	1	18.0	27.0	28.0	29.5
G6	57	1	13.0	23.5	24.0	26.0
G6	65	1	13.5	17.5	22.5	23.0
G6	68	1	11.5	23.0	23.5	28.0
G6	69	1	14.0	24.0	23.0	25.0
G6	71	1	23.0	---	---	---
G6	77	1	14.0	19.5	21.5	23.5
G7	9	2	19.5	30.0	31.0	30.0
G7	10	2	13.5	26.0	29.0	30.5
G7	16	2	12.0	24.0	26.5	27.0
G7	25	2	11.0	26.5	29.0	32.0
G7	30	2	13.0	22.5	24.5	24.0
G7	42	2	14.0	27.0	30.0	30.5
G7	46	2	9.5	15.5	15.5	17.0
G7	47	2	16.5	---	---	---
G7	63	2	18.5	31.5	33.0	34.5
G7	75	2	11.5	23.5	26.5	27.5
G8	4	2	12.0	22.5	25.5	26.5
G8	20	2	12.5	22.0	25.0	25.0
G8	26	2	10.0	23.5	26.5	26.0
G8	51	2	16.0	27.0	30.0	32.0
G8	52	2	17.0	30.0	31.5	29.5
G8	58	2	25.0	---	---	---
G8	61	2	14.0	27.0	29.5	31.0
G8	64	2	12.0	24.5	27.0	27.5
G8	72	2	9.0	20.5	29.5	26.0
G8	74	2	11.5	22.0	---	---

Table 6- Total Faecal Egg Counts

Animal ID	Grp	Plot	Treatment	Day 0	Day 21	Day 42	Day 63	Day 70	Day 84	Day 98	Day 112	Day 126	Day 140	Day 154	Day 168
12	1	G2	controls	0	NS	0	160	720	1560	4000	17000	0	0	NS	0
15	1	G2	controls	0	NS	1360	1640	1560	2680	12120	20800	0	320	9440	40
18	1	G2	controls	0	40	0	760	3480	3920	6920	40	0	1840	40	0
21	1	G2	controls	0	0	NS	120	NS	2640	3040	NS	19280	0	2000	12560
23	1	G2	controls	0	80	40	480	2320	1160	2640	7120	40	0	NS	NS
32	1	G2	controls	0	0	0	400	960	880	1720	2720	4640	0	80	0
33	1	G2	controls	0	0	0	600	1000	1560	3920	10960	0	0	6480	40
38	1	G2	controls	0	0	0	40	440	920	1440	2600	3320	0	0	0
41	1	G2	controls	0	0	80	0	760	960	2440	5880	4520	NS	80	0
80	1	G2	controls	0	0	0	360	600	1520	3520	8720	0	0	6040	0
2	1	G3	controls	0	NS	NS	1840	1680	2160	3000	4600	0	0	1160	3920
7	1	G3	controls	0	40	200	1160	2480	2200	5280	7760	0	0	320	1160
11	1	G3	controls	0	0	80	0	560	640	2360	5400	0	120	2200	2240
29	1	G3	controls	0	0	40	560	760	1400	6120	NS	0	600	760	560
31	1	G3	controls	0	40	0	360	1400	2520	7560	0	0	5160	0	40
40	1	G3	controls	0	0	40	240	520	1360	5440	0	0	7280	NS	NS
50	1	G3	controls	0	0	120	640	1400	2160	2040	11760	40	0	0	800
55	1	G3	controls	0	0	0	80	560	1960	2960	6920	0	0	4880	NS
60	1	G3	controls	0	0	0	200	280	1440	3760	9600	0	0	80	80
78	1	G3	controls	0	0	0	160	NS	1160	1480	8600	0	0	720	1720
6	1	G4	controls	0	0	80	80	280	640	280	560	320	160	80	360
35	1	G4	controls	0	0	0	280	NS	400	760	2800	4520	40	0	440
36	1	G4	controls	0	0	40	80	240	320	80	120	0	480	800	240
37	1	G4	controls	0	0	0	200	1160	1440	1080	2840	3640	9080	0	0

43	1	G4	controls	NS	0	80	600	1240	1480	920	1600	2080	9480	0	160
44	1	G4	controls	0	520	40	760	9200	5680	0	1120	2320	6280	0	80
45	1	G4	controls	0	0	80	0	1440	1880	5200	6920	0	240	40	2240
49	1	G4	controls	0	0	40	360	2440	3400	1800	2560	0	1000	3760	0
62	1	G4	controls	0	0	0	1400	NS	1960	1200	4360	9640	760	0	1040
79	1	G4	controls	0	0	0	NS	NS	NS	NS	NS	NS	NS	NS	NS
1	1	G6	controls	0	80	240	800	1200	6200	7600	80	80	3280	0	0
28	1	G6	controls	0	40	0	200	600	3320	2120	5080	0	0	0	0
34	1	G6	controls	0	0	NS	360	1280	2360	3480	8160	3680	0	0	0
54	1	G6	controls	0	0	0	160	280	720	3720	6000	6400	0	0	0
57	1	G6	controls	0	0	0	120	800	2240	3600	11120	1560	1720	11760	80
65	1	G6	controls	0	0	0	0	400	1400	3880	5280	40	0	0	1720
68	1	G6	controls	0	320	40	600	760	4280	7560	12280	3320	0	320	1920
69	1	G6	controls	0	0	0	720	2720	6760	15600	120	40	17400	0	0
71	1	G6	controls	0	80	0	NS	NS	NS	NS	NS	NS	NS	NS	NS
77	1	G6	controls	0	40	0	40	0	NS	5280	16680	0	0	80	1560
3	2	G1	IVP	0	0	40	200	640	4640	5600	7440	40	1840	5680	12800
17	2	G1	IVP	0	0	0	NS	200	80	520	1080	NS	80	280	880
24	2	G1	IVP	0	80	40	600	1600	2040	1640	3360	1360	680	1840	3440
39	2	G1	IVP	0	0	0	40	0	600	120	1240	1360	0	NS	2000
48	2	G1	IVP	0	0	0	0	40	120	5720	160	160	0	40	240
56	2	G1	IVP	0	0	0	120	200	240	160	640	440	0	80	880
59	2	G1	IVP	0	40	0	40	160	1080	200	680	1000	200	200	840
66	2	G1	IVP	0	0	80	80	160	440	480	2600	640	120	240	1200
70	2	G1	IVP	0	0	0	160	280	320	240	1200	760	0	0	80

73	2	G1	IVP	0	0	0	0	40	240	80	560	240	0	80	440
5	2	G5	IVP	0	0	120	0	40	480	240	560	120	920	640	1160
8	2	G5	IVP	0	0	0	80	NS	640	120	560	320	280	680	680
13	2	G5	IVP	0	0	0	200	400	1040	280	1640	1240	680	1480	920
14	2	G5	IVP	0	0	0	NS	40	160	120	280	160	1000	2400	560
19	2	G5	IVP	0	0	0	40	120	1240	160	160	640	720	1000	640
22	2	G5	IVP	0	40	0	0	280	1360	160	440	1520	760	1480	1720
27	2	G5	IVP	NS	0	80	280	560	1640	200	200	800	2240	6880	4120
53	2	G5	IVP	0	0	0	120	160	1360	720	280	360	960	2160	1920
67	2	G5	IVP	0	40	40	80	80	560	160	120	360	760	1880	0
76	2	G5	IVP	0	0	NS	0	120	360	120	120	160	200	480	0
9	2	G7	IVP	0	0	0	40	640	440	480	520	400	1080	1360	800
10	2	G7	IVP	0	40	0	160	480	1400	760	1640	760	2360	1520	960
16	2	G7	IVP	0	0	0	80	40	920	160	240	280	280	320	760
25	2	G7	IVP	0	0	0	160	400	1480	640	440	760	440	520	320
30	2	G7	IVP	0	0	40	40	280	NS	600	2240	1920	1440	NS	4080
42	2	G7	IVP	0	0	0	0	640	760	840	2120	2720	2200	3240	680
46	2	G7	IVP	0	0	0	40	NS	480	560	280	4320	0	920	2840
47	2	G7	IVP	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
63	2	G7	IVP	0	40	NS	320	840	1240	360	200	320	680	800	880
75	2	G7	IVP	0	0	NS	0	160	160	360	760	1040	1520	1000	1240
4	2	G8	IVP	0	40	40	200	440	1680	800	1360	2360	1680	NS	40
20	2	G8	IVP	0	40	40	1000	1040	2920	800	1320	2720	2000	5200	1000
26	2	G8	IVP	0	0	0	40	200	400	520	760	800	1360	400	1120
51	2	G8	IVP	0	0	0	80	120	1240	160	800	400	600	1000	1040
52	2	G8	IVP	0	0	0	120	720	2320	560	2000	1880	2760	5400	0

58	2	G8	IVP	0	0	0	NS	NS	NS	NS	NS	NS	NS	NS	NS
61	2	G8	IVP	0	160	0	40	120	3440	1080	1120	4800	1080	2240	3160
64	2	G8	IVP	0	0	0	0	80	560	160	760	1480	4160	4120	NS
72	2	G8	IVP	0	0	0	0	40	440	40	480	280	1640	2080	1920
74	2	G8	IVP	0	0	0	120	560	2680	1040	3360	NS	NS	NS	NS

Table 7: Larval Differentiation data

Group	Day	Treatment	Haem	Trich	Ost	Coop	Oes	Total
Lambs	0	Pre - Lambs	2	0	2	0	0	4
Ewes		Pre - Ewes	83	6	10	0	1	100
1	21	Neg. controls	26	0	16	0	0	42
2		Vaccine	30	9	59	2	0	100
1	42	Neg. controls	99	0	1	0	0	100
2		Vaccine	98	0	2	0	0	100
1	63	Neg. controls	95	2	0	3	0	100
2		Vaccine	90	5	3	2	0	100
1	70	Neg. controls	76	4	14	6	0	100
2		Vaccine	72	4	16	8	0	100
1	84	Neg. controls	92	3	3	1	1	100
2		Vaccine	93	2	4		1	100
G1	98	Vaccine	81	1	14	3	1	100
G2		Neg. control	92	2	4	2		100
G3		Neg. control	97	1	1	1		100
G4		Neg. control	82	4	11	3		100
G5		Vaccine	70	11	11	7	1	100
G6		Neg. control	97	0	2	1		100
G7		Vaccine	65	10	17	8		100
G8		Vaccine	50	17	30	2	1	100
G1	112	Vaccine	48	19	31	1	1	100
G2		Neg. control	98	2	0	0	0	100
G3		Neg. control	95	5	0	0	0	100
G4		Neg. control	91	5	3	0	1	100
G5		Vaccine	64	20	16	0	0	100
G6		Neg. control	92	7	1	0	0	100
G7		Vaccine	71	15	14	0	0	100
G8		Vaccine	84	8	8	0	0	100
G1	126	Vaccine	48	34	15	0	3	100
G2		Neg. control	85	14	0	0	1	100
G3		Neg. control	1	1	0	0	0	2
G4		Neg. control	98	0	0	0	2	100
G5		Vaccine	75	18	6	0	1	100
G6		Neg. control	92	2	2	0	4	100
G7		Vaccine	74	22	3	0	1	100
G8		Vaccine	91	8	1	0	0	100
G1	140	Vaccine	100	0	0	0	0	100
G2		Neg. control	89	0	2	0	0	91
G3		Neg. control	84	10	6	0	0	100
G4		Neg. control	92	2	5	1	0	100
G5		Vaccine	50	12	28	9	1	100
G6		Neg. control	99	0	1	0	0	100

G7		Vaccine	48	4	47	1	0	100
G8		Vaccine	56	24	20	0	0	100
G1	154	Vaccine	0	0	0	0	0	0
G2		Neg. control	6	0	0	0	0	6
G3		Neg. control	7	8	0	0	0	15
G4		Neg. control	0	0	0	0	0	0
G5		Vaccine	30	67	3	0	0	100
G6		Neg. control	90	5	5	0	0	100
G7		Vaccine	35	51	14	0	0	100
G8		Vaccine	82	18	0	0	0	100
G1	168	Vaccine	98	0	2	0	0	100
G2		Neg. control	69	27	4	0	0	100
G3		Neg. control	59	38	0	0	3	100
G4		Neg. control	84	14	2	0	0	100
G5		Vaccine	56	41	2	0	1	100
G6		Neg. control	88	11	0	0	1	100
G7		Vaccine	39	48	10	0	3	100
G8		Vaccine	39	58	0	0	3	100

Table 8- Blood Haemoglobin concentrations (g/dl)

Animal ID	Group	Paddock	Treatment	Day 0	Day 21	Day 42	Day 63	Day 70	Day 84	Day 98	Day 112	Day 126	Day 140	Day 154	Day 168
12	1	G2	controls	10.5	---	11.8	10.6	11.5	9.2	7.8	**1.9**	**5.0**	7.5	6.4	8.1
15	1	G2	controls	11.5	---	12.1	10.4	10.7	8.8	6.9	**3.0**	6.9	7.1	**2.6**	7.0
18	1	G2	controls	10.6	---	9.8	9.2	9.2	7.8	**5.8**	8.1	9.5	**3.9**	7.0	9.0
21	1	G2	controls	10.9	---	---	10.2	---	9.2	7.9	6.9	**4.4**	7.4	7.5	*4.9*
23	1	G2	controls	10.3	---	10.9	10.6	10.9	9.0	9.3	**6.3**	9.5	9.7	---	---
32	1	G2	controls	9.2	---	10.2	9.5	10.3	7.8	8.8	6.8	**4.2**	**6.0**	8.0	9.2
33	1	G2	controls	11.5	---	11.8	11.2	12.0	8.2	9.2	**6.1**	9.2	8.8	**4.1**	7.3
38	1	G2	controls	10.8	---	10.7	10.2	10.4	9.0	9.0	7.5	**5.8**	8.3	9.1	8.9
41	1	G2	controls	12.1	---	11.6	11.5	12.4	10.6	11.4	7.9	6.6	**1.8**	**4.7**	7.1
80	1	G2	controls	11.4	---	11.3	10.9	11.0	8.9	7.9	**3.3**	8.6	8.3	**4.6**	8.4
2	1	G3	controls	11.9	---	---	8.5	9.4	7.0	6.7	**2.9**	8.0	9.3	6.7	*5.4*
7	1	G3	controls	11.6	---	11.5	10.6	11.1	9.0	7.9	**4.2**	8.6	9.6	10.2	10.0
11	1	G3	controls	11.3	---	10.6	10.0	10.1	10.4	8.6	**4.7**	7.6	8.3	7.3	7.0
29	1	G3	control	10.7	---	9.9	10.1	10.4	8.9	**5.1**	7.9	8.6	9.1	9.0	9.9

			s												
31	1	G3	controls	9.9	---	11.2	10.6	9.9	8.2	**4.3**	7.6	9.1	**2.7**	6.6	8.3
40	1	G3	controls	11.3	---	11.7	10.8	10.7	9.3	**5.9**	8.1	8.6	**1.4**	---	---
50	1	G3	controls	11.3	---	10.9	9.9	10.4	7.6	6.8	**2.7**	**6.2**	8.3	8.5	8.3
55	1	G3	controls	10.1	---	10.7	9.5	10.5	8.2	7.6	**3.6**	6.9	8.5	**6.4**	8.0
60	1	G3	controls	10.0	---	11.2	10.6	12.3	11.3	8.8	**5.2**	7.1	9.6	10.1	9.8
78	1	G3	controls	8.4	---	9.5	9.5	---	9.6	8.1	**4.8**	7.5	8.4	6.9	*6.3*
6	1	G4	controls	11.5	---	11.4	11.2	11.8	10.2	11.1	12.4	11.1	11.1	11.0	10.6
35	1	G4	controls	12.4	---	11.3	10.9	11.7	10.7	9.8	8.2	**5.6**	7.7	10.1	9.3
36	1	G4	controls	12.7	---	12.9	11.5	12.2	11.5	11.1	11.1	12.0	11.4	11.7	11.3
37	1	G4	controls	10.7	---	11.9	10.9	12.4	10.8	10.8	10.0	8.5	**6.1**	8.1	8.9
43	1	G4	controls	8.3	---	9.8	8.7	9.0	7.8	8.2	7.8	6.8	**5.3**	7.9	8.5
44	1	G4	controls	10.6	---	9.9	9.3	9.0	**5.9**	9.8	8.6	6.7	**4.2**	7.8	9.1
45	1	G4	controls	9.2	---	10.1	9.6	9.6	8.0	6.7	**3.1**	**6.2**	7.8	8.2	*5.3*
49	1	G4	controls	11.1	---	11.5	10.8	10.9	8.7	8.0	**6.2**	8.3	8.7	**4.1**	7.1
62	1	G4	control	11.1	---	11.5	10.8	---	9.5	8.7	7.1	**4.2**	7.7	8.6	8.0

			s												
79	1	G4	controls	10.3	---	11.2	---	---	---	---	---	---	---	---	---
1	1	G6	controls	11.2	---	11.3	10.4	10.0	6.6	**4.8**	9.6	8.5	**4.6**	7.7	8.9
28	1	G6	controls	11.2	---	10.3	9.7	10.5	8.6	7.9	7.1	**5.4**	7.7	9.3	9.9
34	1	G6	controls	11.0	---	11.5	10.5	10.7	9.2	8.2	6.8	**5.3**	9.6	10.1	8.9
54	1	G6	controls	10.8	---	10.6	11.9	10.3	8.8	7.9	7.8	**6.5**	9.6	10.2	10.2
57	1	G6	controls	10.1	---	9.6	10.2	11.3	9.9	9.3	**5.2**	9.1	9.6	**5.9**	8.5
65	1	G6	controls	9.8	---	10.8	10.6	11.8	10.2	9.1	7.4	**4.1**	7.3	9.4	8.7
68	1	G6	controls	8.3	---	11.3	10.1	10.3	8.0	7.2	**2.7**	**6.2**	9.9	9.1	6.6
69	1	G6	controls	9.8	---	10.1	9.0	8.7	6.6	**3.8**	7.0	7.6	**2.6**	**5.8**	7.0
71	1	G6	controls	11.8	---	11.8	---	---	---	---	---	---	---	---	---
77	1	G6	controls	12.2	---	11.3	10.9	11.8	9.3	7.0	**3.2**	**5.9**	9.1	9.7	9.7
3	2	G1	IVP	9.4	11.2	11.9	11.2	11.7	9.8	6.9	**4.8**	8.9	9.5	7.2	*5.5*
17	2	G1	IVP	12.7	12.0	11.5	10.7	11.5	10.1	9.8	10.3	9.8	9.4	10.1	10.4
24	2	G1	IVP	11.6	11.9	12.5	11.4	11.9	10.7	10.8	10.7	9.4	10.3	10.9	10.4
39	2	G1	IVP	10.5	11.4	12.2	10.3	11.4	10.2	11.2	10.4	10.5	10.3	10.8	10.0
48	2	G1	IVP	10.9	11.4	10.6	10.3	11.2	10.4	10.1	10.8	11.1	9.4	10.9	10.2
56	2	G1	IVP	11.8	11.6	10.0	10.3	11.6	10.8	10.4	11.0	10.8	10.6	11.1	10.0

59	2	G1	IVP	10.3	10.8	9.3	9.6	11.5	9.3	10.1	10.7	9.5	7.6	9.3	8.5
66	2	G1	IVP	10.8	11.4	11.1	10.6	11.5	10.7	10.0	9.2	9.2	10.4	10.8	9.7
70	2	G1	IVP	10.5	10.1	11.1	9.7	10.9	9.9	9.8	9.2	9.8	10.1	10.5	10.0
73	2	G1	IVP	12.4	12.6	12.7	11.9	13.1	11.3	11.8	11.9	11.6	12.5	11.6	11.9
5	2	G5	IVP	12.2	12.1	11.5	11.9	13.2	11.7	11.0	11.8	10.1	9.3	10.5	11.5
8	2	G5	IVP	13.1	12.3	11.9	11.2	12.5	10.6	10.6	11.9	10.6	10.5	10.4	10.4
13	2	G5	IVP	12.7	11.4	12.1	11.4	11.7	9.2	9.3	10.0	8.8	7.4	9.2	9.4
14	2	G5	IVP	11.0	10.8	11.2	11.7	11.4	10.3	10.8	11.9	10.3	9.1	9.1	8.5
19	2	G5	IVP	10.7	10.4	10.9	11.2	11.4	10.2	9.9	10.5	9.8	9.5	10.1	10.0
22	2	G5	IVP	11.3	10.7	10.1	9.9	9.8	8.6	8.9	10.3	8.8	8.8	8.7	9.4
27	2	G5	IVP	9.3	10.9	10.2	10.3	9.9	9.4	9.8	10.8	9.1	6.9	**5.9**	*5.4*
53	2	G5	IVP	9.7	10.8	10.8	9.5	9.9	10.1	9.7	10.0	9.4	9.5	9.7	9.5
67	2	G5	IVP	10.6	10.4	9.7	9.3	10.4	8.9	9.2	9.4	8.6	8.6	9.4	10.2
76	2	G5	IVP	11.7	12.0	---	10.8	11.5	9.6	10.4	10.3	9.7	9.6	10.1	9.4
9	2	G7	IVP	13.1	13.1	12.5	11.7	13.3	12.5	11.2	9.3	11.1	10.0	10.6	9.7
10	2	G7	IVP	10.4	10.6	12.2	10.9	10.8	9.6	10.3	10.8	9.2	8.9	8.9	9.1
16	2	G7	IVP	11.8	11.3	10.2	10.2	12.0	11.2	10.5	11.5	10.7	9.7	9.3	9.0
25	2	G7	IVP	10.2	10.8	10.6	10.6	11.1	9.2	9.6	9.3	9.1	8.6	8.9	9.0
30	2	G7	IVP	11.6	11.3	12.0	10.9	11.5	10.3	10.6	10.7	10.5	9.1	8.1	7.7
42	2	G7	IVP	9.8	10.2	11.0	10.7	11.4	9.9	11.3	10.9	9.8	8.1	8.2	8.5
46	2	G7	IVP	9.0	8.8	8.7	9.0	9.3	8.6	8.9	12.2	**6.3**	6.9	6.7	*6.0*
47	2	G7	IVP	9.8	---	---	---	---	---	---	---	---	---	---	---
63	2	G7	IVP	11.2	12.9	11.8	10.3	10.9	9.8	10.1	10.9	10.2	8.8	9.0	9.7
75	2	G7	IVP	10.8	10.6	11.3	11.2	11.0	10.9	10.7	10.2	9.7	9.3	8.6	8.9
4	2	G8	IVP	10.0	11.0	10.9	10.3	11.0	9.1	10.2	9.4	9.1	**6.2**	8.8	10.3
20	2	G8	IVP	10.6	10.3	11.4	11.1	10.8	9.5	10.1	10.3	8.4	7.0	7.2	*6.3*

26	2	G8	IVP	8.7	10.8	9.6	9.8	11.6	10.1	10.6	8.7	9.7	8.3	8.9	8.9
51	2	G8	IVP	12.3	11.3	12.3	11.3	11.9	10.7	11.0	11.3	10.4	8.3	8.9	8.4
52	2	G8	IVP	10.6	10.9	11.0	10.5	10.1	8.5	10.2	10.3	8.1	7.0	**5.4**	7.6
58	2	G8	IVP	13.9	11.7	13.2	---	---	---	---	---	---	---	---	---
61	2	G8	IVP	11.1	11.5	11.0	10.4	11.2	9.7	10.0	10.6	7.7	7.8	8.3	6.8
64	2	G8	IVP	9.5	9.2	10.6	10.8	10.4	9.9	9.8	9.9	9.9	6.8	**5.8**	8.3
72	2	G8	IVP	8.7	9.9	10.8	10.4	10.8	10.5	9.5	10.4	10.4	9.6	9.6	9.5

Table 9. Tracer sheep abomasal worm counts

Kill #1: 12 FEB 13

Sheep ID	Plot ID	Haemonchus spp.	Teladorsagia spp.	Total
107	G1	0	0	0
120	G1	680	60	740
82	G2	460	100	560
103	G2	640	0	640
121	G3	200	0	200
126	G3	4460	100	4560
123	G4	20	0	20
125	G4	0	0	0
102	G5	20	60	80
106	G5	880	80	960
99	G6	20	40	60
112	G6	1020	140	1160
111	G7	0	40	40
128	G7	80	0	80
89	G8	107	0	107
110	G8	40	60	100

Kill #2: 12 MAR 13

Sheep ID	Plot ID	Haemonchus spp.	Teladorsagia spp.	Trichostrongylus	Total
122	G1	6880	20	0	6900
127	G1	3300	380	0	3680
88	G2	5600	0	0	5600
104	G2	2960	0	0	2960
86	G3	1540	20	0	1560
97	G3	4500	280	0	4780
108	G4	220	0	0	220
118	G4	880	20	40	940
93	G5	240	0	0	240
117	G5	1280	0	0	1280
119	G6	280	0	0	280
129	G6	5800	80	0	5880
90	G7	280	60	0	340
109	G7	400	0	0	400
85	G8	380	20	0	400
101	G8	3600	0	0	3600

Kill #3: 9 APR 13

Sheep ID	Plot ID	*Haemonchus spp.*	*Teladorsagia spp.*	Total
83	G1	220	0	220
113	G1	2740	80	2820
95	G2	1020	0	1020
105	G2	6640	60	6700
114	G3	100	20	120
116	G3	2080	40	2120
84	G4	220	0	220
100	G4	6340	40	6380
96	G5	280	0	280
115	G5	160	0	160
98	G6	12340	120	12460
124	G6	11280	380	11660
81	G7	160	0	160
94	G7	1420	0	1420
87	G8	100	0	100
91	G8	920	60	980

Table 10. Rainfall data from Armidale tree nursery weather station
Summer 2012-2013 daily rainfall (mm).

Day of Month	Nov	Dec	Jan	Feb	Mar	Apr
1st		0	*0*	*0*	*12.8*	*0*
2nd		0	*0*	*14.6*	*26*	*0*
3rd		23.6	*0*	*0*	*5.8*	*0*
4th		3.8	*0*	*0*	*4.4*	*1*
5th		0	0	*0*	*0.8*	*0.6*
6th	*0*	0	0	*0.6*	*0*	*0*
7th	*0.2*	0	*0*	0	*0.4*	*0*
8th	*0*	0	*0*	0	*0*	*3.2*
9th	*4*	1	*0*	0	0.2	*0*
10th	*10.2*	15.4	*0*	*0*	0	*0*
11th	*1.2*	0.4	*0*	*10*	0	*0*
12th	*0*	0.4	*0*	*0.2*	0.6	*0*
13th	*0*	0.4	*3.2*	*0.2*	0.6	*0*
14th	*0.6*	0	*10.4*	*0*	*0*	*0*
15th	*2*	0	*0.4*	*0*	*0*	*0*
16th	*0*	0	*0*	*0*	*0*	*0*
17th	*2.2*	0	*0*	*0.8*	*0*	*1.8*
18th	*20.2*	0	*0*	*0*	*0*	*0*
19th	*0*	0	*0*	*0.4*	*0*	*0*
20th	*0*	10.8	*47.8*	*1.4*	*0*	*0*
21st	*0*	0	*4.4*	*1.6*	*2.6*	*0*

22nd	*0*	0.4	*0*	*2.8*	*0*	*0*
23rd	*0*	0.4	*1.2*	*42.8*	*0*	*0*
24th	*0*	0	*24.2*	*0.4*	*0*	*0*
25th	*4.8*	1.4	*0*	*0*	*0*	*0*
26th	*0*	30.4	*0*	*10.2*	*0*	*0*
27th	*0*	0.6	*13.8*	*3.6*	*0*	*0*
28th	*0.8*	*2.6*	*46.6*	*0*	*0*	*0*
29th	*2*	*1.4*	*10*		*1.2*	*0*
30th	*0.2*	*0*	*0*		*0*	*0*
31st			*0*		*0*	
Total monthly rain	**48.4**	**93**	**162**	**89.6**	**55.4**	**6.6**

Table 11- Group 2 (Vaccinates) ELISA titres

		Day after V1										
Paddock	**Animal #**	**21**	**42**	**63**	**70**	**84**	**98**	**112**	**126**	**140**	**154**	**168**
G1	3	0	162	5,401	5,394	4,367	6,343	5,242	5,429	6,938	6,223	6,276
G1	17	1,660	2,864	8,240	7,386	6,047	10,018	8,599	7,125	11,161	8,665	10,302
G1	24	27	2,984	8,642	7,463	7,458	12,109	9,911	8,046	10,406	8,665	10,790
G1	39	3	6,902	8,518	6,992	6,587	8,132	7,317	6,377	8,612	7,442	7,725
G1	48	0	6,872	37,883	11,914	40,949	122,175	174,308	94,749	239,004	157,891	173,652
G1	56	86	5,844	8,734	7,018	6,469	14,313	10,863	7,640	13,494	9,284	11,536
G1	59	3	4,371	6,836	5,881	5,972	9,686	8,860	6,805	6,101	5,343	5,539
G1	66	5	2,251	10,000	4,085	3,228	7,043	7,104	5,216	10,397	6,633	6,150
G1	70	2	5,265	5,522	5,824	4,993	8,672	7,629	6,813	17,364	10,202	9,870
G1	73	65	3,945	8,301	8,024	6,391	21,889	10,533	9,018	26,462	12,256	14,217
G5	5	49	3,742	7,325	5,697	5,663	10,790	10,343	6,822	13,799	9,019	9,692
G5	8	39	3	7,873	6,911	6,306	27,953	31,431	20,555	88,400	32,776	55,003
G5	13	49	4,521	5,956	6,908	4,716	7,259	7,122	6,007	7,677	6,425	6,155
G5	14	15	3,521	5,239	4,567	4,184	8,622	10,224	6,951	6,684	6,227	6,810
G5	19	46	2,761	8,674	7,536	7,565	61,754	32,909	15,014	56,614	26,319	28,000
G5	22	91	2,464	6,857	6,100	4,822	10,647	7,664	6,788	10,657	8,566	10,109
G5	27	0	0	4,960	-	6,531	42,908	27,078	16,751	12,359	9,477	10,238
G5	53	2	3,133	-	5,550	5,111	8,120	7,855	6,404	5,927	5,217	5,835
G5	67	230	7,107	7,063	8,009	7,232	38,431	10,530	8,239	29,143	11,775	11,371
G5	76	82	-	771	654	1,234	29,868	14,570		36,885	14,535	10,773
G7	9	1,974	1,311	5,797	5,578	5,181	13,829	15,350	11,866	26,805	25,146	37,071
G7	10	43	2,132	7,362	7,096	5,843	8,749	7,634	6,114	6,763	6,228	8

G7	16	54	4,868	4,885	4,509	4,370	13,668	19,182	9,760	28,348	16,119	16,928
G7	25	213	2,784	926	1,052	1,758	12,513	14,160	8,102	18,929	10,355	10,298
G7	30	52	2,823	6,813	5,645	5,474	7,252	8,585	7,364	8,265	6,795	7,616
G7	42	-	588	4,900	4,827	3,308	9,777	9,847	6,565	9,966	7,262	7,663
G7	46	0	1,817	4,380	3,440	2,975	7,423	7,149	5,258	7,831	6,222	6,092
G7	47	=	=	-	-	0						-
G7	63	46	2,476	9,519	5,909	3,129	9,930	8,922	6,743	17,866	10,536	12,182
G7	75	4	2,673	4,735	4,335	3,075	9,845	10,322	9,305	10,636	8,311	7,965
G8	4	0	696	4,167	4,643	2,371	6,386	7,458	5,240	7,393	6,536	6,297
G8	20	178	5,414	18,866	10,653	8,940	14,213	8,967	7,349	10,357	7,349	7,495
G8	26	0	1,573	6,331	6,161	5,913	8,626	5,736	7,746	9,617	7,650	7,261
G8	51	387	3,638	7,421	31,337	5,711	9,474	10,045	6,506	16,090	9,228	9,020
G8	52	951	338	2,732	1,787	739	6,961	6,566	4,878	5,818	4,504	3,962
G8	58	1	2,029	-	-	0						-
G8	61	206	1,128	9,032	7,932	5,273	9,664	10,513	6,821	11,757	7,123	7,460
G8	64	0	3,072	4,945	8,322	6,647	11,631	8,561	6,512	7,611	5,717	6,164
G8	72	240	1,732	8,301	4,831	3,693	10,594	8,616	7,381	28,535	10,618	12,960
G8	74	0	2,168	5,055	6,060	3,254	7,884	7,601	6,720			-

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Table 12- Group 1 Antibody Titres

Day after V1		84	168
Paddock	Animal #	Ab titre	
G2	12	4	148
G2	15	1	91
G2	18	68	446
G2	21	37	1,997
G2	23	39	-
G2	32	106	2,902
G2	33	1	2,343
G2	38	59	2,652
G2	41	1,219	909
G2	80	2	355
G3	2	24	50
G3	7	1	2,074
G3	11	27	4,261
G3	29	0	1,911
G3	31	0	159
G3	40	821	-
G3	50	14	2,090
G3	55	20	749
G3	60	1	928
G3	78	0	4,354
G4	6	0	218
G4	35	682	1,933
G4	36	102	2,252
G4	37	5,154	3,266
G4	43	179	135
G4	44	1,060	283
G4	45	35	1,536
G4	49	1	1,764
G4	62	0	430
G4	79		-
G6	1	0	267
G6	28	0	160

G6	34	0	95
G6	54	71	1,628
G6	57	148	1,051
G6	65	16	7
G6	68	0	1,058
G6	69	96	1,603
G6	71		-
G6	77	29	3,977

APPENDIX 4

Statistcal Outputs

Comparison of the Haemonchus egg counts averaged from day 70 till end of trial in vaccine and control plots using the T test.

Table Analyzed	From d70mean epg by plot
Column B	Control Hc
vs.	vs.
Column A	Vaccine Hc
Unpaired t test	
P value	0.0079
P value summary	**
Significantly different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=3.912 df=6
How big is the difference?	
Mean ± SEM of column A	748.3 ± 156.8 N=4
Mean ± SEM of column B	2649 ± 459.9 N=4
Difference between means	1901 ± 485.9
95% confidence interval	712.0 to 3090
R squared	0.7184
F test to compare variances	
F,DFn, Dfd	8.606, 3, 3
P value	0.1104
P value summary	ns
Significantly different? (P < 0.05)	No

No of sheep drenched or not drenched – from day 84 of the trial (see table 1)

Table Analyzed	Day 84 table 1
Fisher's exact test	
P value	**0.0115**
P value summary	*
One- or two-tailed	Two-tailed

Statistically significant? (alpha<0.05)	**Yes**		
Data analyzed	drenched	not drenched	Total
Control	7	31	38
Vaccinated	0	38	38
Total	7	69	76

Table Analyzed	Day 98 table 1		
Fisher's exact test			
P value	**< 0.0001**		
P value summary	****		
One- or two-tailed	Two-tailed		
Statistically significant? (alpha<0.05)	**Yes**		
Data analyzed	drenched	not drenched	Total
Control	17	21	38
Vaccinated	0	38	38
Total	17	59	76

Table Analyzed	Day 112 table 1		
Fisher's exact test			
P value	**0.0003**		
P value summary	***		
One- or two-tailed	Two-tailed		
Statistically significant? (alpha<0.05)	**Yes**		
Data analyzed	drenched	not drenched	Total
Control	14	24	38
Vaccinated	1	37	38
Total	15	61	76

Table Analyzed	Day 126 table 1		
Fisher's exact test			
P value	**0.0067**		
P value summary	**		
One- or two-tailed	Two-tailed		
Statistically significant? (alpha<0.05)	**Yes**		
Data analyzed	drenched	not drenched	Total
Control	10	28	38
Vaccinated	1	37	38
Total	11	65	76

Table Analyzed	Day 126 table 1		
Fisher's exact test			
P value	**0.0284**		
P value summary	*		
One- or two-tailed	Two-tailed		
Statistically significant? (alpha<0.05)	**Yes**		
Data analyzed	drenched	not drenched	Total
Control	8	30	38
Vaccinated	1	37	38
Total	9	67	76

Table Analyzed	Total drenched after day 70		
Fisher's exact test			
P value	**< 0.0001**		
P value summary	****		
One- or two-tailed	Two-tailed		
Statistically significant? (alpha<0.05)	**Yes**		
Data analyzed	drenched	not drenched	Total
Control	10	294	304
Vaccinated	61	243	304
Total	71	537	608

Table Analyzed	No of sheep never requiring a drench		
Fisher's exact test			
P value	**< 0.0001**		
P value summary	****		
One- or two-tailed	Two-tailed		
Statistically significant? (alpha<0.05)	**Yes**		
Data analyzed	drenched	not drenched	Total
Control	36	2	38
Vaccinated	8	30	38
Total	44	32	76

9.3 Appendix 3 – CSIRO report of vaccine trial in yearlings

CSIRO Livestock Industries

Field test of Moredun *Haemonchus* vaccine efficacy in Merino yearlings.

Sponsor:	Name: Address:	Julie Fitzpatrick Moredun Group Director Moredun Institute The Moredun Group Pentlands Science Park Bush Loan Penicuik Midlothian Scotland, UK
Sponsor Monitor and Representative:	Name: Quals: Address: Phone: E-mail:	David Smith BVMS, PhD The Moredun Group Pentlands Science Park Bush Loan Penicuik Midlothian Scotland, UK +44 (0)131 445 6131 David.Smith@moredun.ac.uk
Investigator:	Name: Quals: Address: Phone: E-mail:	Malcolm Knox BSc(Hons), MSc, PhD CSIRO Livestock Industries F.D. McMaster Laboratory-Chiswick Armidale NSW 2359 02 6776 1440 Malcolm.Knox@csiro.au

Field test of MRI Haemonchus vaccine efficacy in Merino yearlings.

SUMMARY

Haemonchus contortus is the major nematode pathogen in high rainfall areas where small ruminants are produced. An effective vaccine against this pathogen is highly desirable. During the summer of 2011-2012 the efficacy of the Moredun Research Institute *Haemonchus* vaccine was assessed in 40 young "Elite" Merino lambs in the field during the period post weaning. The results were highly encouraging because over the course of the trial the vaccine reduced Haemonchus egg output by 82% on average. See Field trial 2 report in the original dossier for full details

The object of present trial was to evaluate the same vaccine over the 2012-2013 summer in the same sheep which were now yearlings.

A group of 30 of these sheep (20 female and 10 castrate male) were run with 30 sex and age-matched previously unvaccinated controls so that all experienced the same natural challenge. Because they had been vaccinated before, an anamnestic response was expected after the vaccinates received their first immunisation (see the supplementary Trial 1 report in the previously submitted dossier) and the interval between each boost was fixed at 6 weeks.

Compared to the controls, this vaccine regime successfully reduced egg counts as well as the consequent degree of anaemia and the number of precautionary drenches needed, indicating that a course of 4 injections was sufficient to substantially protect hoggets (which had previously been vaccinated as lambs) against Haemonchosis.

Field test of MRI Haemonchus vaccine efficacy in Merino hoggets.

Objective

To assess the efficacy of "Barbervax", the Moredun Research Institute *Haemonchus* vaccine, for Merino hoggets over the high risk period of exposure to this parasite. If positive, the results will extend the scope of the registration package for this commercial product to yearling sheep.

Justification

Haemonchus contortus is the major nematode pathogen in high rainfall areas of Australia where small ruminants are produced. An effective vaccine against this pathogen is highly desirable. Previous work established that Barbervax induced high levels of protection in lambs on five different Australian farms during the summer of 2011-2012, but its protective ability for older sheep remained untested.

Compliance

This study was conducted in accordance with the approved protocol and with CSIRO Standard Operating Procedures, unless otherwise stated, and the study objectives were achieved.

Test Site

Animal phase	***Laboratory phase***
CSIRO Livestock Industries	CSIRO Livestock Industries
Arding Field Station	F.D. McMaster Laboratory- Chiswick
Armidale NSW 2350	Armidale NSW 2350

Antibody analyses

Moredun Research Institute

Edinburgh, UK

Study Dates

Start date (Animal Phase): 4 December 2012

Finish date (Animal phase): 24 April 2013

Finish date (laboratory phase): 4 October 2013

Study Design

Thirty Elite Merino yearlings (20 female and 10 castrate male) which had been vaccinated with Barbervax during the 2011-2012 summer formed the Vaccine group for this study. The Control group contained 30 sex and age-matched sheep randomly selected from the same flock at Arding, after excessively heavy or light "outliers" had been removed. All trial animals were run together as a single mob.

Four doses of vaccine were given at 6 week intervals over the trial, the first on December 4th and the last on April 11. Faecal samples were collected from individual sheep at 2 week intervals from December 4 until April 24 for faecal worm egg count (FEC) estimation. On the same days, blood was collected by jugular venepuncture into 6 mL sodium heparin vacutainers (BD Ltd, Australia) for haemoglobin concentration estimation (using the Haemocue method) and for plasma and serology. Liveweights were recorded at Days 2, 71 and 146 of the trial. Laboratory personnel performing FEC and haemoglobin analysis were blinded as to treatment groups.

If at any time during the trial, the blood haemoglobin concentration of any animal was <8.5 g/100mL (equivalent PCV 25%) or the FEC was >15,000, that sheep was treated immediately with Tri Gard (or an alternative short-acting anthelmintic effective against *Haemonchus*) at the manufacturer's recommended dose rate. The animal remained with the trial flock after such treatment.

Investigational and control products

m. Investigational Veterinary Product (IVP):

Name:	BarberVax	Batch No.:	HCD220311C-004
Composition:	*Haemonchus* antigen and saponin adjuvant	Expiry Date:	October 2013
Dose Rate:	5µg antigen and 1mg saponin	WHP:	12 months

n. Source:

WormVax Laboratory
Animal Health Laboratory
DAFWA
444 Albany Highway
Albany, W.A. 6330

o. Storage: Refrigerated at 4°C until use.

p. Safety: A MSDS was not provided by the Sponsor. The IVP was administered using a specially designed safety vaccine gun to protect against accidental injection.

q. Assays: A Certificate of Analysis was not provided for the IVP.

r. Drug Disposal: All remaining IVP was retained at CSIRO pending disposal advice from the Sponsor.

Treatment

Treatment administration: Vaccinations were delivered sub-cutaneously into the neck of the lambs using a 1 mL Simcro Securus Veterinary Injector (Simcro Animal Health Delivery Systems, New Zealand).

Treatment frequency: On four occasions at Weeks 0, 6, 12 and 18.
Dose: 1 mL per lamb.

Schedule of events

Date	Day	Activity
20 November	-14	Identify and bring together Trial hoggets on B Lane Plots at Arding. Drench with 8 mL TriGuard.
23 November	-11	Welfare check
26 November	-8	Welfare check
29 November	-5	Welfare check
4 December	0	Week 0 - FEC and blood samples from hoggets, assign to groups and Vaccinate (V1) 30 hoggets. Move to pasture plots in G Lane.
6 December	2	Record liveweights of trial hoggets
11 December	7	Welfare check
14 December	10	Welfare check
17 December	13	Welfare check
18 December	14	Week 2 - FEC and blood samples from hoggets. Treat hoggets with Pro-Guard backline to prevent flystrike.
21 December	17	Welfare check
24 December	20	Welfare check
28 December	24	Welfare check
31 December	27	Welfare check
3 January	30	Week 4 - FEC and blood samples from hoggets.
7 January	34	Welfare check
10 January	37	Welfare check
14 January	41	Welfare check

16 January	43	Week 6 - FEC and blood samples from hoggets. Vaccinate (V2) 30 hoggets.
18 January	45	Welfare check. Drench hogget 7007 12 mL HatTrick
21 January	48	Welfare check
25 January	52	Welfare check
29 January	56	Welfare check
30 January	57	Week 8- FEC and blood samples from hoggets.
4 February	62	Welfare check
8 February	66	Welfare check
11 February	69	Welfare check.
13 February	71	Week 10 - FEC and blood samples from hoggets. Liveweights recorded.
14 February	72	Welfare check
15 February	73	Welfare check
18 February	76	Welfare check
19 February	77	Welfare check
22 February	80	Welfare check
25 February	83	Welfare check
27 February	85	Week 12 - FEC and blood samples from hoggets. Vaccinate (V3) 30 hoggets.
28 February	86	Drench hoggets 7006, 7007, 7036, 7047, 7109, 7115, 7117, 7127, 7168, 7239, 7303, 7313, 7358, 7365, 7405, 7417, 7419, 7545, 7677, 7705 with 8 mL TriGuard.
1 March	87	Welfare check
4 March	90	Welfare check
6 March	92	Welfare check
11 March	97	Welfare check.
13 March	99	Week 14 - FEC and blood samples from hoggets.
14 March	100	Drench hoggets 7122, 7239, 7268, 7322, 7441, 7705 with 8 mL TriGuard.
15 March	101	Welfare check
18 March	104	Welfare check
20 March	106	Welfare check
21 March	107	Welfare check
25 March	111	Welfare check

27 March	113	Week 16 - FEC and blood samples from hoggets.
28 March	114	Drench hoggets 7007, 7709 with 8 mL TriGuard.
2 April	119	Welfare check
5 April	122	Welfare check
8 April	125	Welfare check.
11 April	128	Week 18 - FEC and blood samples from hoggets. Vaccinate (V4) 30 hoggets.
12 April	129	Drench hogget 7113 with 8 mL TriGuard.
15 April	132	Welfare check
17 April	134	Welfare check
19 April	136	Welfare check
22 April	139	Welfare check
24 April	141	Week 20 - FEC and blood samples from hoggets. All trial hoggets drenched 5 mL Zolvix.
29 April	146	Hogget liveweights recorded. Treat hoggets 7191, 7405, 7612, 7659 for flystrike. Hoggets returned to Elite hogget flock.

Animal Management

Animal Welfare: Study animals were managed similarly and with due regard for their welfare. Animals were observed at least twice weekly for health problems according to AEC requirements. Animals were handled in compliance with CSIRO AEC 12/25 approved 18/10/12, and any applicable local regulations.

Health Management: Any health problems or adverse events that occurred during the study were recorded (see Study schedule above).

Housing: Routine management practices were followed. All trial animals had *ad-lib* access to pasture consisting of rye, phalaris, clover and native grass species. Potable water was supplied *ad-lib*.

Animal disposal: All remaining animals were returned to the CSIRO flocks on Arding at the conclusion of the study.

Study Procedures

Trial Log: All scheduled and unscheduled events during the study were recorded.

Plasma Sample Storage, Transfer and Disposal: Replicate 1 and 2 samples were stored in separate temperature logged freezers at approximately -20^{o}C until delivery on ice-bricks to Veterinary Health Research, Armidale for onward dispatch to the

Moredun Research Institute, Edinburgh. Replicate 2 plasma samples will be held in frozen storage (-20^{o}C) at CSIRO until disposal is approved by the study sponsor.

Assessment of Effects

Sheep liveweights: Animals were weighed at Days 2, 71 and 146 and individual animal liveweights were recorded. Animal weigh scales were checked pre- and post-weighing with calibrated test weights. Liveweights were compared between groups to determine treatment effects, if any, and are detailed in the results section of this report.

Haemoglobin concentration: Blood haemoglobin concentration from individual lamb whole blood was measured using the Hemocue 201 Hb Analyser. Changes during the study were compared between groups to determine treatment effects, if any, and are detailed in the results section of this report.

Faecal worm egg counts and larval differentiations: Faecal samples were collected at intervals outlined above. Faecal samples were individually labelled with the animal ID. Individual faecal worm egg counts and group bulk larval differentiation were performed. Faecal worm egg counts and larval differentiation were compared to determine treatment effects, if any, and are detailed in the results section of this report.

Note: where an animal received a salvage drench at any point throughout the study, the subsequent FEC sample collected within 14 days of the salvage drench was excluded from group mean FEC calculations.

Blood antibody analyses: Blood samples were processed for collection of plasma samples on the day of collection. Samples were individually labelled with the study number, animal number, study date and day, sample type. Frozen (-20^{o}C) plasma samples were forwarded to Moredun for anti-vaccine antibody titre analysis at completion of the study. Results of analyses were compared to determine treatment effects, if any, and are detailed in the results section of this report.

Statistical Analyses

Faecal egg counts, blood haemoglobin concentrations and bodyweights were compared between groups by the t test, whereas the number of precautionary drenches was compared by Fisher's exact test. The faecal egg counts were log transformed prior to analysis.

Results

Deaths

No sheep died during the trial.

Haemonchus Egg Counts

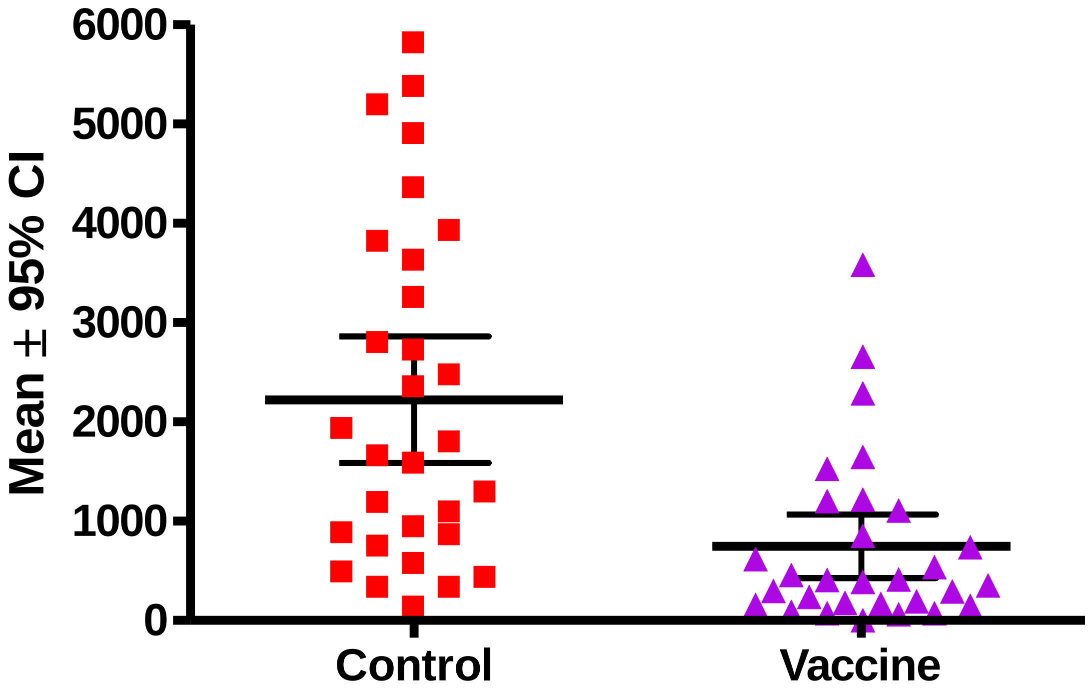

Figure 1. Overall effect of the vaccine on *Haemonchus* egg output of hoggets between 4 December 2012 and 24 April 2013.

Haemonchus egg counts were significantly reduced in the vaccinated sheep compared to the controls as shown in Figure 1 above and Table 1 below. However, four "non-responders" (defined as a vaccinated animal with a mean egg count greater than the 95% lower confidence limit of the control group) were identified.

Table 1	**Day**	**vaccinates**	**controls**	**%efficacy**	**P (log +1 data)**	
V1	**0**	0.0	0.0	_	–	
	14	0.0	0.0	_	–	
	30	106.8	450.0	76.3	2.48E-06	Sig
V2	**43**	665.6	1750.0	62.0	0.002626	Sig
	57	553.6	2186.7	74.7	3.24E-06	Sig
	71	186.0	1687.8	89.0	1.56E-08	Sig
V3	**85**	1156.3	6020.5	80.8	5.86E-06	Sig
	99	677.4	2052.2	67.0	0.022134	Sig
	113	764.7	1895.8	59.7	0.032169	Sig
V4	**128**	1828.2	1903.3	3.9	0.560146	NS
	141	1517.2	3763.3	59.7	0.2178	NS
	Mean	**677.8**	**1973.6**	**63.7**		

(the earliest the vaccine could have an effect is from day 14)

c. Anti-vaccine antibody status at the beginning of the trial

At the start of this study ELISA titres in the unvaccinated sheep were very low and remained so throughout the trial (Table 2, Fig 2). In contrast, those of the vaccinated sheep were substantially greater at trial start and by two weeks after their first vaccination mean titres increased more than 6-fold (Table 2, Fig 2).

Table 2. Group mean antibody titres at the start of the trial

	Control day 0	Vaccinated day 0	Vaccinated day 14
mean	31	6,194	38,575
SE	12	1,385	20,705

d. Kinetics of and relationships between the parameters studied over the course of the trial.

The kinetics of total and *Haemonchus* specific egg counts were very similar throughout the trial because *Haemonchus* eggs always represented some 90% of the strongyle egg populations of both groups (Fig 2 and Table A4).

Egg counts in December were very low, but rose in the controls to a mean of about 2,000 epg by late January and reached a temporary peak of some 6,000 epg a month later (Fig 2). Mean vaccinate egg counts were consistently and significantly lower during this part of the trial, never exceeding 1000 epg except on day 85. However, mean vaccinate counts did increase somewhat towards the end of the trial and were not significantly different from control values on the last two sampling days (Fig 2).

Individual antibody titres were highly variable in the vaccinated sheep. Two weeks after each boost a temporary peak in response was observed which declined until the next immunisation day (Table A6). Mean titres remained above 10,000 throughout the trial (Fig 2).

Mean blood haemoglobin concentrations fell at similar rates in both groups during January (Fig 3). Then Vaccinate haemoglobin means fluctuated around 100g/L during February and the first half of March, but by April they had returned to about 110g/L, a concentration close to that at the start of the trial. Meanwhile, mean control haemoglobins fell to below 90g/L by the end of February, but these also recovered, attaining 105 to 110 g/L by the end of the trial (Fig 3).

Up to mid February (Day 71) a few sheep in both groups possessed haemoglobin concentrations below the 85g/L threshold (Table A2) and parallel PCV estimations confirmed this anaemia was genuine rather than due to a fault with the Haemacue machine. Since these animals had relatively low egg counts and seemed strong and healthy clinically, it was decided that the cause of anaemia was unlikely to be *Haemonchus* and so no precautionary treatments were given until Day 85 when Control egg counts reached their peak (Fig 3). Significantly fewer precautionary treatments were given to the vaccinates over the course of the trial (Table 4).

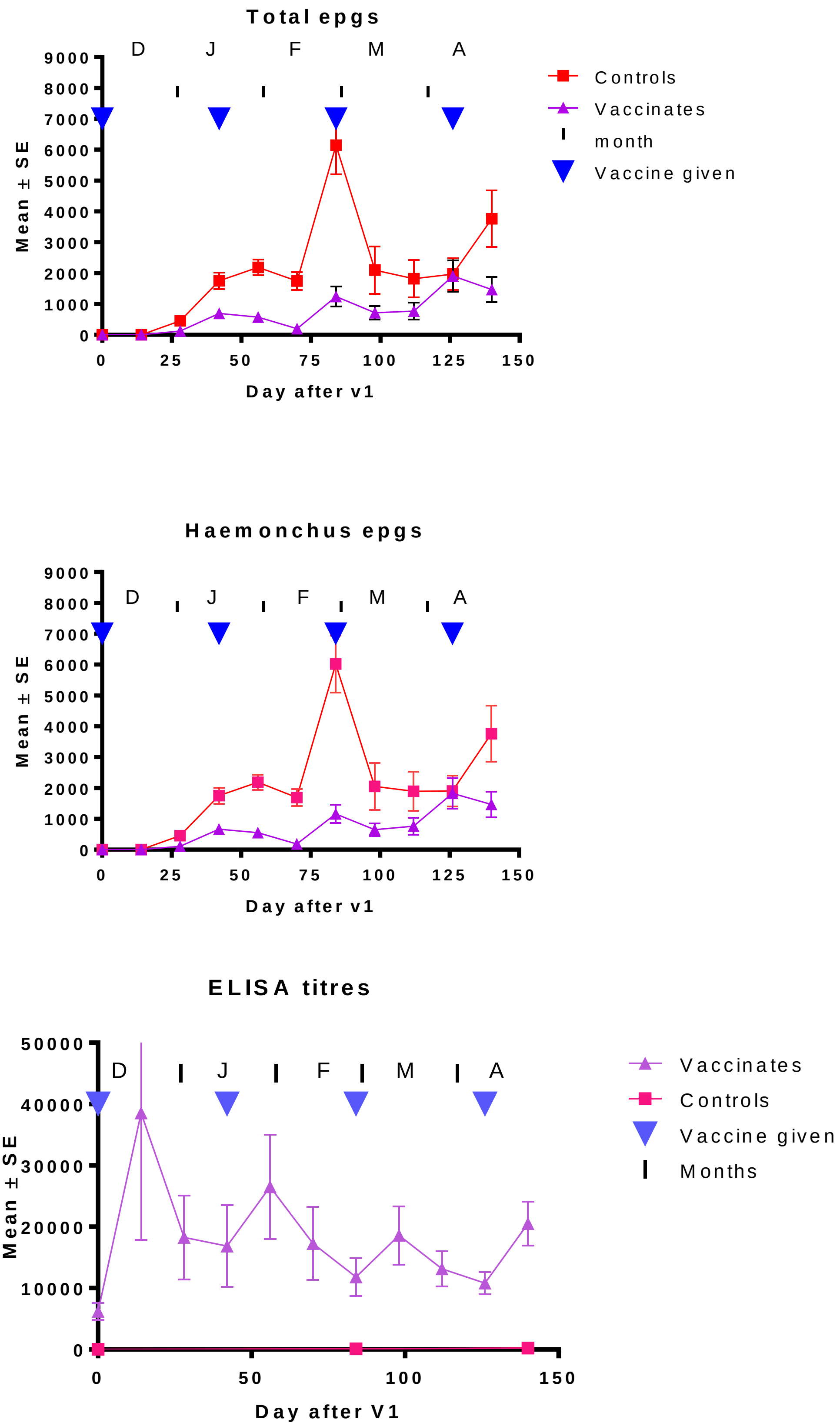

Figure 2. Kinetics of interventions, group mean total egg counts, *Haemonchus* specific egg counts and the anti vaccine antibody response.

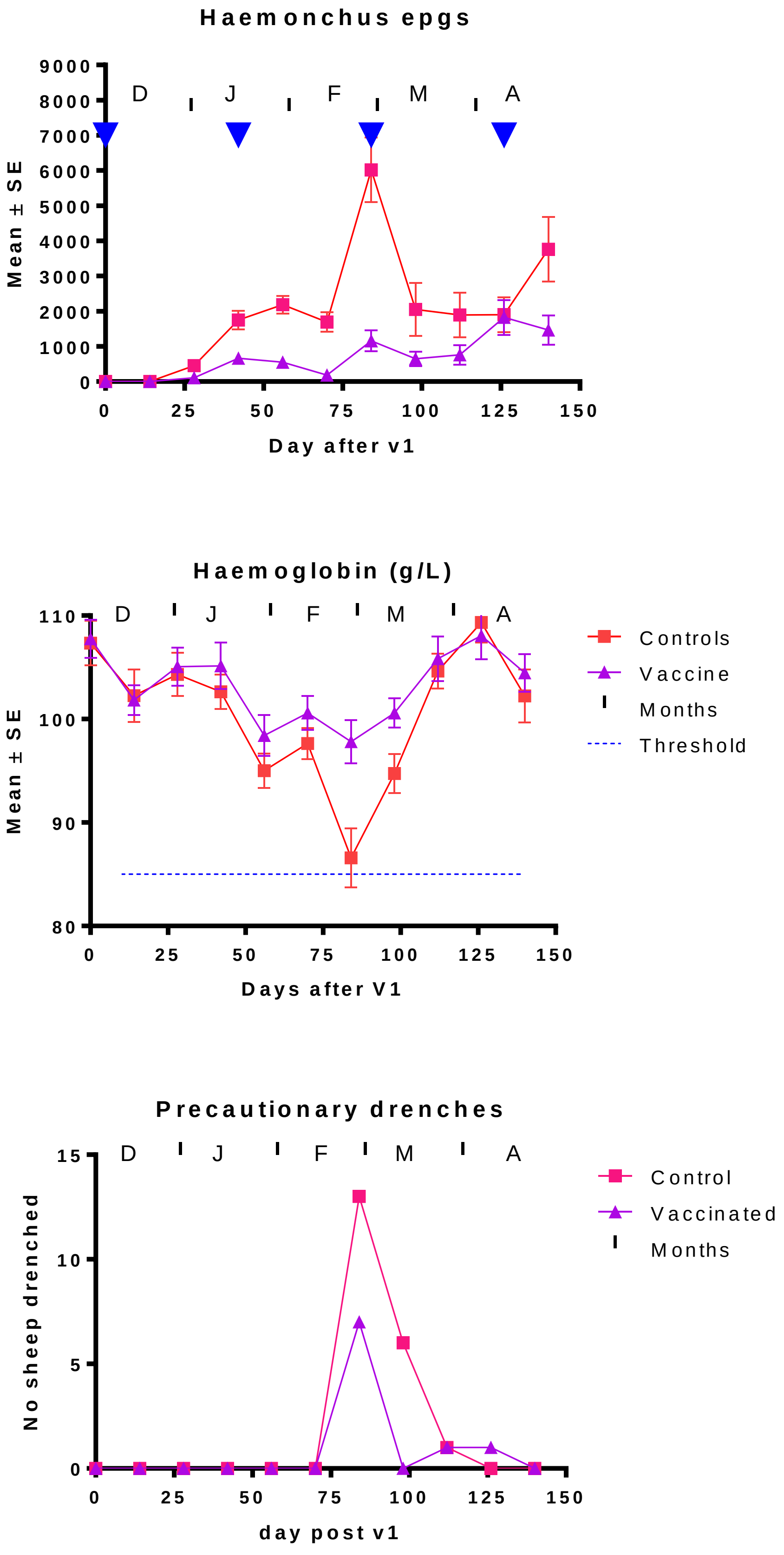

Figure 3. Kinetics of the *Haemonchus* specific egg counts in relation to blood haemoglobin concentrations and to precautionary drenching.

Table 4. Number of precautionary drenches given to vaccinated and control sheep

Day post V1	**Control**	**Vaccinated**	**Fishers exact 2-tail P**
0	0	0	
14	0	0	
30	0	0	
43	0	0	
57	0	0	
71	0	0	
85	13	7	NS
99	6	0	0.01
113	1	1	NS
128	0	1	NS
141	0	0	
Total	**20**	**9**	**<0.01**

Bodyweights

The sheep were weighed at the intervals detailed in Section 9 of the Protocol. Group mean bodyweights are presented in the Figure below. No significant differences ($p<0.05$, t test) were detected between the groups at any stage of trial.

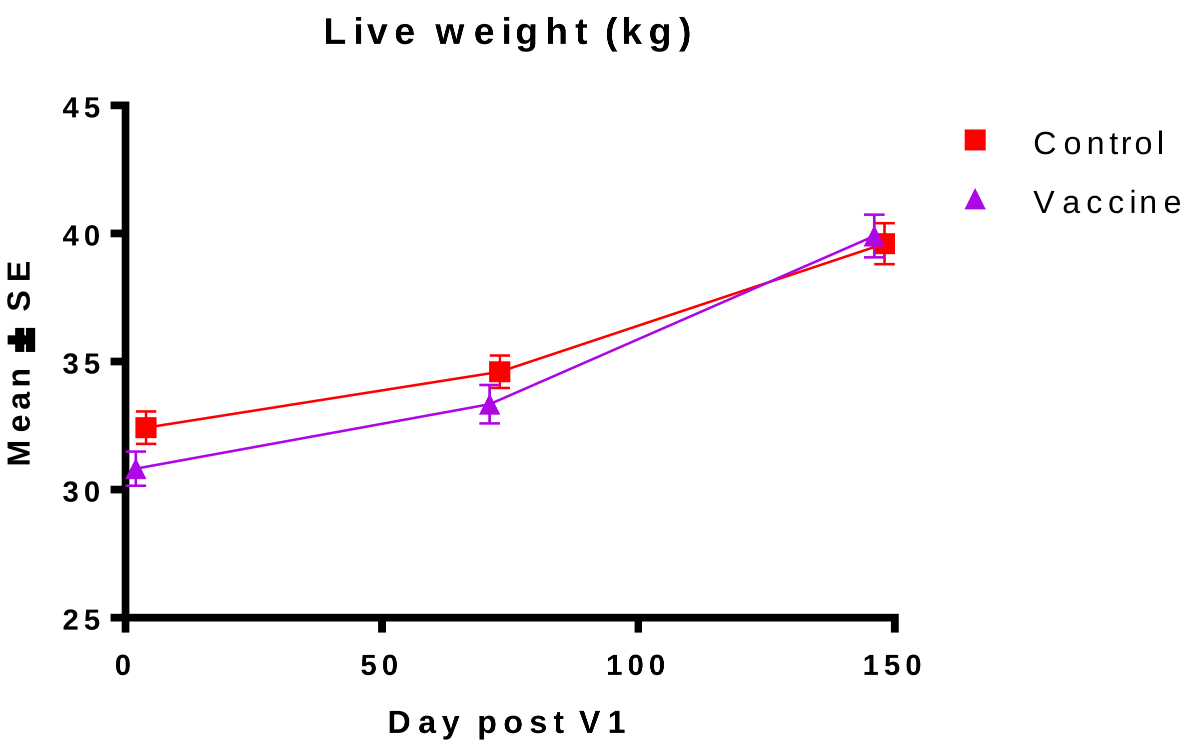

Fig 4. Sheep weights during the course of the trial

Discussion/Conclusions

In this study Barbervax was evaluated in yearling Merinos which had received a course of the same vaccine as lambs the previous summer. Their faecal egg counts, blood haemoglobin concentrations and serological status were compared with those of age matched unvaccinated controls. For simplicity, and to ensure equal exposure of both groups to the natural challenge infection, all sheep grazed together throughout the trial.

Barbervax substantially protected the vaccinated sheep over the course of the trial. It reduced *Haemonchus* egg output by about 64% on average and mitigated the consequent anaemia, hence significantly reducing the number of precautionary treatments the vaccinates required compared to the controls.

The first immunisation stimulated a clear anamnestic serological response in the vaccinates and 3 further boosts 6 weeks apart maintained high antibody titres and protective immunity from mid December to late April, the period of greatest *Haemonchus* risk in New England. Thus hoggets which had received a course of Barbervax the previous summer required four not five immunisations to prevent Haemonchosis in their second year.

Appendix 1 Tabulated and raw data.

Table A.1: Allocation of trial yearlings to treatment groups and liveweights (kg) on Days 0, 70 and 140 of the trial. Group C were the unvaccinated controls and Group V were the vaccinated sheep.

Tag Prefix	Tag No.	Sex	Group	06/12/2012	13/02/2013	29/04/2013
2011A	7109	F	C	35.5	38.5	43
2011A	7117	F	C	36	38	42.5
2011A	7122	F	C	34.5	38	45.5
2011A	7146	F	C	31.5	34.5	43
2011A	7155	F	C	33.5	36	37
2011A	7239	M	C	27.5	28.5	37
2011A	7254	M	C	30.5	35.5	39.5
2011A	7260	M	C	34.5	38	43.5
2011A	7268	M	C	30.5	35	44.5
2011A	7303	M	C	30.5	33.5	39
2011A	7313	F	C	27.5	29	35
2011A	7322	M	C	30.5	32.5	37
2011A	7353	F	C	32.5	32.5	39
2011A	7358	F	C	33	35	40
2011A	7365	F	C	35.5	38	40.5
2011A	7371	F	C	39.5	40.5	47
2011A	7405	F	C	29.5	32.5	33.5
2011A	7417	F	C	30	28	38.5
2011A	7419	F	C	30.5	32	36.5
2011A	7441	F	C	28.5	32	38.5
2011A	7524	M	C	34.5	36.5	43
2011A	7544	M	C	38	40	46
2011A	7545	M	C	28	31	36.5
2011A	7572	M	C	36	39	45
2011A	7602	F	C	30.5	33	31.5
2011A	7612	F	C	31.5	34	35
2011A	7659	F	C	28.5	29.5	29
2011A	7677	F	C	39.5	36	41.5
2011A	7705	F	C	36.5	39.5	43
2011A	7709	F	C	28	32.5	38
2011A	0118	F	V	34.5	33.5	42.5
2011A	7006	M	V	27.5	30.5	42.5
2011A	7007	M	V	29	32.5	40
2011A	7014	M	V	25.5	28.5	39
2011A	7036	M	V	31	36	43.5
2011A	7042	M	V	28.5	35	42.5
2011A	7045	M	V	30	35	44.5
2011A	7047	M	V	33	37.5	46

2011A	7070	M	V	26.5	31.5	41
2011A	7072	M	V	29.5	34	43
2011A	7086	M	V	22.5	26.5	34.5
2011A	7099	F	V	34	37	38
2011A	7101	F	V	36	38.5	41.5
2011A	7110	F	V	23.5	23	30
2011A	7113	F	V	30.5	32	33.5
2011A	7115	F	V	36.5	39	41
2011A	7116	F	V	33.5	35.5	39.5
2011A	7120	F	V	33	34	41.5
2011A	7126	F	V	29.5	30	35
2011A	7127	F	V	29	30	34
2011A	7137	F	V	31	33	42.5
2011A	7163	F	V	34	35	41
2011A	7165	F	V	33	37.5	45
2011A	7166	F	V	31	34.5	41
2011A	7168	F	V	31	32.5	38
2011A	7178	F	V	39.5	42.5	48
2011A	7180	F	V	31	33	39
2011A	7182	F	V	32.5	34.5	41
2011A	7186	F	V	30.5	33.5	41.5
2011A	7191	F	V	28	25	27

These sheep were suffering from fly strike when weighed.

Table A.2 Haemoglobin concentration (mg/mL) for trial yearlings. Those below the 85 mg/ml threshold highlighted in yellow.

			weeks	0	2	4	6	8	10	12	14	16	18	20
				V1			**V2**			**V3**			**V4**	
				04-Dec	18-Dec	03-Jan	16-Jan	31-Jan	13-Feb	27-Feb	13-Mar	27-Mar	10-Apr	24-Apr
Tag Prefix	**Tag No.**	**Sex**	**Group**	**0**	**14**	**28**	**42**	**56**	**70**	**84**	**98**	**112**	**126**	**140**
2011A	7109	F	C	129	118	96	102	96	89	80	90	101	103	106
2011A	7117	F	C	87	96	87	93	88	95	68	102	101	92	84
2011A	7122	F	C	110	114	101	101	95	100	94	79	98	113	108
2011A	7146	F	C	112	116	95	117	105	107	115	114	116	123	124
2011A	7155	F	C	110	106	120	115	106	112	108	113	119	119	122
2011A	7239	M	C	104	97	73	93	105	98	63	82	103	117	105
2011A	7254	M	C	108	111	109	100	89	98	95	107	107	115	103
2011A	7260	M	C	97	96	110	102	91	99	102	106	111	116	113
2011A	7268	M	C	89	85	101	114	102	95	88	81	94	109	107
2011A	7303	M	C	98	98	111	87	83	87	71	91	97	100	108
2011A	7313	F	C	100	98	114	104	102	113	84	103	115	120	112
2011A	7322	M	C	102	109	128	123	120	107	95	82	112	134	128
2011A	7353	F	C	112	104	105	98	87	99	90	97	108	103	99
2011A	7358	F	C	109	107	117	107	80	91	72	99	108	116	80
2011A	7365	F	C	108	98	104	107	93	98	79	94	98	95	83
2011A	7371	F	C	71	77	104	108	91	100	90	100	106	114	102
2011A	7405	F	C	110	93	100	93	84	83	84	98	108	100	93
2011A	7417	F	C	113	107	107	106	91	88	53	89	98	107	94
2011A	7419	F	C	106	100	102	92	93	98	79	92	97	92	84
2011A	7441	F	C	107	113	92	99	91	91	87	74	108	120	92
2011A	7524	M	C	111	105	113	108	104	98	114	110	119	112	120
2011A	7544	M	C	120	109	106	99	90	91	88	99	103	102	96
2011A	7545	M	C	108	106	102	94	87	81	49	88	94	91	75
2011A	7572	M	C	103	64	107	87	96	108	96	98	109	117	116
2011A	7602	F	C	107	84	113	99	96	107	100	95	97	97	84
2011A	7612	F	C	128	115	112	106	95	99	96	106	124	114	110
2011A	7659	F	C	122	141	81	114	107	105	100	89	110	114	101
2011A	7677	F	C	102	95	105	104	98	95	84	96	100	101	98

2011A	7705	F	C	121	108	98	94	80	89	81	82	100	104	97
2011A	7709	F	C	116	98	116	113	105	108	93	86	78	120	123
2011A	0118	F	V	112	108	111	120	99	105	111	108	114	131	118
2011A	7006	M	V	98	104	95	83	80	88	82	95	94	93	107
2011A	7007	M	V	113	105	72	67	72	78	84	107	59	115	128
2011A	7014	M	V	110	103	109	111	98	97	109	99	103	113	103
2011A	7036	M	V	110	102	106	104	101	97	81	93	104	88	93
2011A	7042	M	V	115	95	97	101	84	94	94	101	111	104	108
2011A	7045	M	V	104	102	114	106	109	104	94	91	96	99	92
2011A	7047	M	V	114	104	101	91	84	85	80	89	108	102	103
2011A	7070	M	V	88	101	98	89	88	90	94	88	96	112	99
2011A	7072	M	V	110	108	99	101	97	92	91	100	103	112	108
2011A	7086	M	V	104	109	110	105	95	94	97	98	104	103	111
2011A	7099	F	V	135	125	119	109	108	113	112	96	103	109	102
2011A	7101	F	V	99	95	105	113	96	100	95	90	108	87	97
2011A	7110	F	V	109	108	95	109	91	106	96	90	99	97	97
2011A	7113	F	V	87	88	114	119	112	104	98	106	105	78	91
2011A	7115	F	V	110	101	96	93	91	110	85	98	114	114	101
2011A	7116	F	V	93	88	107	111	111	109	114	104	115	120	113
2011A	7120	F	V	113	111	99	103	96	96	94	106	110	109	104
2011A	7126	F	V	109	104	111	111	112	102	110	109	115	112	116
2011A	7127	F	V	111	94	104	105	90	97	78	94	96	92	80
2011A	7137	F	V	120	100	94	104	114	115	111	108	114	109	97
2011A	7163	F	V	107	102	124	132	118	117	117	118	120	132	128
2011A	7165	F	V	95	101	104	103	94	96	92	104	110	105	106
2011A	7166	F	V	115	105	110	115	102	107	110	107	113	117	109
2011A	7168	F	V	119	103	112	115	108	113	85	104	123	109	101
2011A	7178	F	V	97	89	116	112	90	95	106	110	115	117	106
2011A	7180	F	V	109	95	108	116	105	105	98	93	98	102	100
2011A	7182	F	V	108	98	110	92	103	106	115	115	118	123	106
2011A	7186	F	V	99	93	116	107	101	104	104	99	109	113	105
2011A	7191	F	V	120	114	96	107	103	99	97	98	98	125	105

Table A.3 Faecal worm egg counts (eggs/g faeces) for trial yearlings

Precautionary drenches during the trial : Hb <85mg/mL yellow background, or FEC>15000 pink background.

Tag Prefix	Tag No.	Sex	Group	day post v1	04-Dec	18-Dec	03-Jan	16-Jan	31-Jan	13-Feb	27-Feb	13-Mar	27-Mar	10-Apr	24-Apr
					0	**14**	**28**	**42**	**56**	**70**	**84**	**98**	**112**	**126**	**140**
2011A	7109	F	C		0	0	0	100	300	300	1400		200	300	1800
2011A	7117	F	C		0	0	200	3000	4500	1300	9600		1000	9900	14800
2011A	7122	F	C		0	0	200	800	200	100	700	1000		0	0
2011A	7146	F	C		0	0	200	900	1400	700	700	100	0	0	300
2011A	7155	F	C		0	0	0	100	1000	0	100	0	100	0	0
2011A	7239	M	C		0	0	700	1500	2700	2200	17000			1400	5400
2011A	7254	M	C		0	0	700	1000	1100	200	400	700	700	300	600
2011A	7260	M	C		0	0	200	2100	3900	800	800	300	100	400	200
2011A	7268	M	C		0	0	100	900	900	1000	2600	900		100	300
2011A	7303	M	C		0	0	1200	2800	3100	1300	3900		1900	100	0
2011A	7313	F	C		0	0	400	400	2200	700	6500		0	100	500
2011A	7322	M	C		0	0	600	1000	1800	2200	9700	9400		100	800
2011A	7353	F	C		0	0	300	0	900	800	1300	0	0	0	0
2011A	7358	F	C		0	0	1500	3700	3900	5700	13200		900	2700	8000
2011A	7365	F	C		0	0	600	1700	2100	3100	15800		3100	7500	13200
2011A	7371	F	C		0	0	100	2200	3200	2000	4600	700	500	2800	2000
2011A	7405	F	C		0	0	600	4400	2600	100	1100		200	500	300
2011A	7417	F	C		0	0	1900	6400	4300	7200	12400		2700	6300	7600
2011A	7419	F	C		0	0	300	2600	1800	2300	7900		1800	4600	3400
2011A	7441	F	C		0	0	1400	3600	3400	3600	7600	8000		800	4600
2011A	7524	M	C		0	0	200	900	1500	1200	4000	200	600	200	700
2011A	7544	M	C		0	0	400	1900	2200	1500	3300	700	3500	2500	7600
2011A	7545	M	C		0	0	400	2400	5000	2200	17200		2900	7800	14800
2011A	7572	M	C		0	0	300	1200	1400	1300	4100	1000	1000	1000	1700
2011A	7602	F	C		0	0	400	300	1300	700	2700	1400	6300	4100	15400
2011A	7612	F	C		0	0	0	1200	800	1800	2300	200	200	1000	1200
2011A	7659	F	C		0	0	200	1000	1500	1800	9500	2400	100	0	300

2011A	7677	F	C		0	0	0	700	500	900	7500		3400	2600	6800
2011A	7705	F	C		0	0	300	2700	4200	2500	5900			0	0
2011A	7709	F	C		0	0	100	1000	1900	2700	10500	8600	14300		600
2011A	0118	F	V		0	0	0	200	400	100	100	0	0	0	0
2011A	7006	M	V			0	100	2200	2500	1400	3100		200	300	1700
2011A	7007	M	V			0	400	3400	0	0	0		0		0
2011A	7014	M	V		0	0	100	0	200	100	400	700	400	700	600
2011A	7036	M	V		0	0	0	400	600	700	2100		400	5200	1000
2011A	7042	M	V		0	0	0	500	500	0	0	0	200	700	100
2011A	7045	M	V		0	0	500	2500	1600	300	5900	3500	7100	12400	3400
2011A	7047	M	V		0	0	0	1000	200	0	0		0	300	0
2011A	7070	M	V		0	0	900	2000	1800	0	300	0	0	1400	1400
2011A	7072	M	V		0	0	0	0	100	0	1000	100	0	500	200
2011A	7086	M	V		0	0	200	800	200	400	1000	1800	2000	5400	900
2011A	7099	F	V		0	0	0	0	400	0	300	600	300	1400	1300
2011A	7101	F	V		0	0	0	1100	800	300	1700	3600	4800	6300	8800
2011A	7110	F	V		0	0	200	400	400	0	700	400	1400	2800	2600
2011A	7113	F	V		0	0	0	200	400	100	0	0	0	1200	
2011A	7115	F	V		0	0	0	400	1800	0	2000		0	600	400
2011A	7116	F	V		0	0	0	400	200	0	100	200	0	0	0
2011A	7120	F	V		0	0	0	0	100	100	400	400	0	100	0
2011A	7126	F	V		0	0	0	300	500	0	600	1000	1200	500	800
2011A	7127	F	V		0	0	0	200	200	200	6100		900	2400	4400
2011A	7137	F	V		0	0	0	600	200	0	0	800	500	1000	700
2011A	7163	F	V		0	0	0	0	200	0	0	0	0	0	0
2011A	7165	F	V		0	0	0	300	100	300	700	0	0	600	600
2011A	7166	F	V		0	0	0	200	100	700	1800	300	200	200	1000
2011A	7168	F	V		0	0	700	1200	900	900	5200		1000	5900	5600
2011A	7178	F	V		0	0	100	600	700	0	0	0	0	0	400
2011A	7180	F	V		0	0	0	0	0	0	0	0	300	100	500
2011A	7182	F	V		0	0	400	1000	1400	0	300	0	0	200	0
2011A	7186	F	V		0	0	0	200	700	0	0	900	1600	2900	200
2011A	7191	F	V		0	0	0	700	100	400	3500	2100	600	2300	7400

Table Analyzed	**Total no of drenches given to each group**		
Fisher's exact test			
P value	**0.0092**		
P value summary	**		
One- or two-tailed	Two-tailed		
Statistically significant? (alpha<0.05)	Yes		
Data analyzed	drenched	not drenched	Total
Control	20	10	30
Vaccinated	9	21	30
Total	29	31	60

Table A.4 Larval differentiations from trial hogget cultures

Date	Day	Treatment	Haem	Trich	Ost	Coop	Oes	Total
Jan 3		Control	100	0	0	0	0	100
		Vaccine	89	11	0	0	0	100
Jan 16		Control	100	0	0	0	0	100
		Vaccine	96	4	0	0	0	100
Jan 31		Control	100	0	0	0	0	100
		Vaccine	96	4	0	0	0	100
Feb 13		Control	97	3	0	0	0	100
		Vaccine	93	7	0	0	0	100
Feb 27		Control	98	2	0	0	0	100
		Vaccine	93	7	0	0	0	100
Mar 13		Control	98	2	0	0	0	100
		Vaccine	95	5	0	0	0	100
Mar 27		Control	100	0	0	0	0	100
		Vaccine	96	4	0	0	0	100
Apr 10		Control	100	0	0	0	0	100
		Vaccine	99	0	1	0	0	100
Apr 24		Control	100	0	0	0	0	100
		Vaccine	100	0	0	0	0	100

Table A.5 Yearlings Log (Haemonchus egg counts + 1)

				Days after V1								
Tag Prefix	**Tag No.**	**Sex**	**Group**	**28**	**42**	**56**	**70**	**84**	**98**	**112**	**126**	**140**
2011A	7109	F	C	0.00	2.00	2.48	2.47	3.14		2.30	2.48	3.26
2011A	7117	F	C	2.30	3.48	3.65	3.10	3.97		3.00	4.00	4.17
2011A	7122	F	C	2.30	2.90	2.30	1.99	2.84	2.99		0.00	0.00
2011A	7146	F	C	2.30	2.95	3.15	2.83	2.84	2.00	0.00	0.00	2.48
2011A	7155	F	C	0.00	2.00	3.00	0.00	2.00	0.00	2.00	0.00	0.00
2011A	7239	M	C	2.85	3.18	3.43	3.33	4.22			3.15	3.73
2011A	7254	M	C	2.85	3.00	3.04	2.29	2.59	2.84	2.85	2.48	2.78
2011A	7260	M	C	2.30	3.32	3.59	2.89	2.89	2.47	2.00	2.60	2.30
2011A	7268	M	C	2.00	2.95	2.95	2.99	3.41	2.95		2.00	2.48
2011A	7303	M	C	3.08	3.45	3.49	3.10	3.58		3.28	2.00	0.00
2011A	7313	F	C	2.60	2.60	3.34	2.83	3.80		0.00	2.00	2.70
2011A	7322	M	C	2.78	3.00	3.26	3.33	3.98	3.96		2.00	2.90
2011A	7353	F	C	2.48	0.00	2.95	2.89	3.11	0.00	0.00	0.00	0.00
2011A	7358	F	C	3.18	3.57	3.59	3.74	4.11		2.95	3.43	3.90
2011A	7365	F	C	2.78	3.23	3.32	3.48	4.19		3.49	3.88	4.12
2011A	7371	F	C	2.00	3.34	3.51	3.29	3.65	2.84	2.70	3.45	3.30
2011A	7405	F	C	2.78	3.64	3.42	1.99	3.03		2.30	2.70	2.48
2011A	7417	F	C	3.28	3.81	3.63	3.84	4.08		3.43	3.80	3.88
2011A	7419	F	C	2.48	3.42	3.26	3.35	3.89		3.26	3.66	3.53
2011A	7441	F	C	3.15	3.56	3.53	3.54	3.87	3.89		2.90	3.66
2011A	7524	M	C	2.30	2.95	3.18	3.07	3.59	2.29	2.78	2.30	2.85
2011A	7544	M	C	2.60	3.28	3.34	3.16	3.51	2.84	3.54	3.40	3.88
2011A	7545	M	C	2.60	3.38	3.70	3.33	4.23		3.46	3.89	4.17
2011A	7572	M	C	2.48	3.08	3.15	3.10	3.60	2.99	3.00	3.00	3.23
2011A	7602	F	C	2.60	2.48	3.11	2.83	3.42	3.14	3.80	3.61	4.19
2011A	7612	F	C	0.00	3.08	2.90	3.24	3.35	2.29	2.30	3.00	3.08
2011A	7659	F	C	2.30	3.00	3.18	3.24	3.97	3.37	2.00	0.00	2.48
2011A	7677	F	C	0.00	2.85	2.70	2.94	3.87		3.53	3.42	3.83
2011A	7705	F	C	2.48	3.43	3.62	3.38	3.76		0.00	0.00	0.00
2011A	7709	F	C	2.00	3.00	3.28	3.42	4.01	3.93	4.16	0.00	2.78
2011A	0118	F	V	0.00	2.29	2.59	1.97	1.97	0.00	0.00	0.00	0.00

2011A	7006	M	V	1.95	3.32	3.38	3.11	3.46	0.00	2.29	2.47	3.23
2011A	7007	M	V	2.55	3.51	0.00	0.00	0.00		0.00	0.00	0.00
2011A	7014	M	V	1.95	0.00	2.29	1.97	2.57	2.82	2.59	2.84	2.78
2011A	7036	M	V	0.00	2.59	2.76	2.81	3.29		2.59	3.71	3.00
2011A	7042	M	V	0.00	2.68	2.68	0.00	0.00	0.00	2.29	2.84	2.00
2011A	7045	M	V	2.65	3.38	3.19	2.45	3.74	3.52	3.83	4.09	3.53
2011A	7047	M	V	0.00	2.98	2.29	0.00	0.00			2.47	0.00
2011A	7070	M	V	2.90	3.28	3.24	0.00	2.45	0.00	0.00	3.14	3.15
2011A	7072	M	V	0.00	0.00	1.99	0.00	2.97	1.98	0.00	2.70	2.30
2011A	7086	M	V	2.25	2.89	2.29	2.57	2.97	3.23	3.28	3.73	2.95
2011A	7099	F	V	0.00	0.00	2.59	0.00	2.45	2.76	2.46	3.14	3.11
2011A	7101	F	V	0.00	3.02	2.89	2.45	3.20	3.53	3.66	3.80	3.94
2011A	7110	F	V	2.25	2.59	2.59	0.00	2.81	2.58	3.13	3.44	3.42
2011A	7113	F	V	0.00	2.29	2.59	1.97	0.00	0.00	0.00	3.08	
2011A	7115	F	V	0.00	2.59	3.24	0.00	3.27		0.00	2.77	2.60
2011A	7116	F	V	0.00	2.59	2.29	0.00	1.97	2.28	0.00	0.00	0.00
2011A	7120	F	V	0.00	0.00	1.99	1.97	2.57	2.58	0.00	2.00	0.00
2011A	7126	F	V	0.00	2.46	2.68	0.00	2.75	2.98	3.06	2.70	2.90
2011A	7127	F	V	0.00	2.29	2.29	2.27	3.75		2.94	3.38	3.64
2011A	7137	F	V	0.00	2.76	2.29	0.00	0.00	2.88	2.68	3.00	2.85
2011A	7163	F	V	0.00	0.00	2.29	0.00	0.00	0.00	0.00	0.00	0.00
2011A	7165	F	V	0.00	2.46	1.99	2.45	2.81	0.00	0.00	2.77	2.78
2011A	7166	F	V	0.00	2.29	1.99	2.81	3.22	2.46	2.29	2.30	3.00
2011A	7168	F	V	2.80	3.06	2.94	2.92	3.68		2.98	3.77	3.75
2011A	7178	F	V	1.95	2.76	2.83	0.00	0.00	0.00	0.00	0.00	2.60
2011A	7180	F	V	0.00	0.00	0.00	0.00	0.00	0.00	2.46	2.00	2.70
2011A	7182	F	V	2.55	2.98	3.13	0.00	2.45	0.00	0.00	2.30	0.00
2011A	7186	F	V	0.00	2.29	2.83	0.00	0.00	2.93	3.19	3.46	2.30
2011A	7191	F	V	0.00	2.83	1.99	2.57	3.51	3.30	2.76	3.36	3.87

Table A.6 Antibody titres

Group	Tag #	Animal #	V1 Day 0	Day 14	Day 28	V2 Day 42	day 57	Day 70	V3 Day 84	Day 98	Day 112	V4 Day 127	Day 140
	118	31	45294	627558	205530	202550	236112	179570	97781	141325	92682	58270	108103
	7006	32	3978	6149	5811	5766	6618	5914	5101	7031	5683	5696	6832
	7007	33	2383	4935	3552	3240	13990	10600	7608	18910	11658	9572	24238
	7014	34	5040	19415	13254	8926	12255	9859	8304	12985	8623	7916	15913
	7036	35	4074	18375	7951	6871	10822	8203	6951	9393	8169	6325	14346
	7042	36	5742	13686	10286	9596	16445	12164	9632	18542	12636	8887	32783
	7045	37	933	7779	5266	4587	7068	5823	4912	6542	5609	4910	7554
	7047	38	3981	9047	7919	6261	8851	6848	5725	7509	6379	6318	12282
	7070	39	6084	7989	7625	6673	9846	7593	6669	12343	8618	7907	13185
	7072	40	4207	8750	7116	6753	9704	7445	6864	8941	6969	6486	9921
	7086	41	3685	9845	7347	6845	9744	8085	6538	7191	6153	5765	10197
Vaccinates	7099	42	5500	12054	9828	9302	11212	11369	9963	14519	12766	10905	24887
	7101	43	3481	6939	6063	5731	7087	6292	5693	7717	6943	5711	7310
	7110	44	2201	6591	5908	5379	10984	7891	7704	8981	8042	6991	10621
	7113	45	6071	88802	60125	35743	56477	19652	18836	30322	20603	15661	22550
	7115	46	6278	25550	11550	9294	17273	9574	8708	12110	9486	10988	15101
	7116	47	7809	19240	11895	11054	52767	19424	14244	21270	15827	17635	46693
	7120	48	5306	8951	7623	7814	7572	7203	8111	9781	8366	7737	9500
	7126	49	6143	15639	12345	11374	18337	12354	9967	14784	9735	9139	14337
	7127	50	3790	10596	6689	6391	12398	6876	6581	6910	5941	5554	7379
	7137	51	4645	9416	7125	7016	10068	9030	7758	9720	7918	7333	10140
	7163	52	5576	25986	12426	11797	12491	9328	8331	10461	8651	7942	12413
	7165	53	4933	9309	7808	8541	9803	7481	6784	10021	7655	7382	12085
	7166	54	5672	18470	10089	9488	13680	10474	8648	10447	8339	7515	11099

	7168	55	4495	11463	7974	9365	20045	10086	8119	14247	10987	9320	14292
	7178	56	4459	10176	7481	7025	6303	5915	5472	12562	8989	7885	15288
	7180	57	10651	103583	51427	58050	134360	69098	26510	71273	32181	29542	37689
	7182	58	4394	22534	14266	10488	27337	14165	11085	20019	14655	12149	32868
		59	4203	8346	7277	6260	10904	9316	7537	10094	11760	8560	13925
		60	4821	10081	7979	6923	14844	10531	8304	12587	12777	8634	42717
mean			**6194**	**38575**	**18251**	**16837**	**26513**	**17272**	**11815**	**18618**	**13160**	**10821**	**20541**
sem			1385	20705	6836	6687	8516	5969	3068	4760	2908	1853	3606
	7109	1	1						7				26
	7117	2	4						435				196
	7122	3	0						10				21
	7146	4	6						0				46
	7155	5	155						503				1099
	7239	6	201						0				28
	7254	7	0						32				75
	7260	8	101						35				186
	7268	9	9						45				155
	7303	10	0						0				0
	7313	11	35						523				1307
Controls	7322	12	0						89				218
	7353	13	0						1				0
	7358	14	0						0				0
	7365	15	3						0				475
	7371	16	0						0				327
	7405	17	4						56				471
	7417	18	0						0				70
	7419	19	19						135				888
	7441	20	0						0				31

	7524	21	111	0	0
	7544	22	1	30	201
	7545	23	1	0	24
	7572	24	4	0	0
	7602	25	4	0	4
	7612	26	0	1	10
	7659	27	251	66	729
	7677	28	1	0	0
		29	20	796	427
		30	4	36	26
mean			**31**	**93**	**235**
sem			12	36	64

Table A6

WEATHER DATA
Data from Australian Bureau of Meteorology

Armidale airport- total monthly rainfall (mm)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2012											*47.6*	*80.2*
2013	*160.6*	*92*	*52.4*	*3.2*								

9.4 Appendix 4. VHR report of yearling vaccine trial conducted near Dundee, NSW

Study Title: A field study to test the efficacy of a Haemonchus vaccine in yearling sheep during times of high parasite challenge, North New England district NSW, Australia.

Study No.:	**MIHO 2898**	**Sponsor Study No.:**	N/A
Version No.:	**1**	**Version Date:**	July 2013
Author:			

Sponsor:	Name: Address:	Julie Fitzpatrick Moredun Group Director Moredun Institute The Moredun Group Pentlands Science Park Bush Loan Penicuik Midlothian Scotland, UK
Sponsor Monitor and Representative:	Name: Address: Phone: E-mail:	David Smith The Moredun Group Pentlands Science Park Bush Loan Penicuik Midlothian Scotland, UK +44 (0)131 445 6131 David.Smith@moredun.ac.uk
Investigator:	Name: Quals: Address: Phone: E-mail:	Henry Chambers BLivSc (Hons) Veterinary Health Research Pty. Ltd. Trevenna Road, Armidale, NSW 2350 02 6770 3200 hchambers@vhr.com.au

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STUDY INVESTIGATOR

COMPLIANCE STATEMENT

I, the undersigned, hereby declare that the report is a complete, true and accurate representation of the study and its results.

This study was conducted in accordance with the approved Protocol and with VHR Standard Operating Procedures (see Appendix 3), unless otherwise stated, and the study objectives were achieved. The study was conducted in compliance with:

- VICH GL9 Good Clinical Practice (June 2000)
- APVMA Vet MORAG – Efficacy and target animal safety (Vol. 3, Part 8, 01 Jul 07)

There were no deviations from Protocol or any other circumstances considered to have affected the outcome of the study.

Signed: ____

Jessica McLeod B. LivSc (Hons)
Study Investigator

Date: ____

QUALITY ASSURANCE STATEMENT

Inspections were made by the Quality Assurance Unit of the various phases of the study described in this report. The date inspections were carried out and reported to the Investigator and to facility management are given below:

Inspection Date	Inspection Type	Phase Inspected	QA Auditor	Inspection Report issued

This report has been audited by the Quality Assurance Unit and is considered to be an accurate description of the methods and procedures used during the conduct of the study, and an accurate reflection of the raw data.

Signed: ______________________________

Leonora J Pearson DipRQA
Quality Assurance Manager

Date: ______________________________

18. Objective

To confirm the field efficacy of a *Haemonchus* vaccine in yearling sheep against *Haemonchus contortus* in the West New England region of New South Wales, Australia. Data from this study may be used to support product registration.

19. Justification

Internal parasites of sheep are usually controlled by the strategic use of anthelmintic drugs. In the warmer, wetter parts of Australia Haemonchus contortus usually dominates the species of gut nematode parasites found in sheep. Haemonchus resistant to many of the commonly used anthelmintics are widespread in Australia and many other parts of the world. A vaccine to control Haemonchus would reduce the dependence on anthelmintics and be of great benefit to sheep producers.

20. Compliance

The study complied with the following national and international standards:

VICH GL9 Good Clinical Practice (issued June 2000)
APVMA Vet MORAG – Efficacy and target animal safety (Vol. 3, Part 8, 01 Jul 07)

21. Test sites

Animal Phase:

Laboratory Phase:
Veterinary Health Research P/L
Colin Blumer Animal Health Laboratory
Trevenna Road
Armidale NSW 2350 Australia

Analytical Phase:
Moredun Institute
Pentlands Science Park
Bush Loan
Penicuik, Midlothian EH26 0PZ
SCOTLAND, UK

22. Study dates

Start date (animal phase): 29 nov 2012
Finish date (animal phase): 16 may 2013
Finish date (laboratory phase): 26 october 2013

23. Study design

g. **Experimental Unit:** The experimental unit was the individual animal.

Animal Model: Thirty Merino yearlings which had been vaccinated with Barbervax during the 2011-2012 summer formed the Vaccine group for this study. The Control group contained 30 age matched sheep randomly selected from the same flock, after excessively heavy or light "outliers" had been removed. All trial animals were run together as a single mob.

h. . All study animals were harbouring a natural mixed infection of *Haemonchus, Trichostrongylus, Teladorsagia, Cooperia* and *Oesophagostomum* species, predominantly *Haemonchus contortus.*

i. Inclusion Criteria: Animals were selected for the study if they met the criteria outlined in section 10 below.

j. Exclusion and Removal Criteria: No animals were excluded or removed from the study.

k. Allocation: Thirty male or female Merino yearlings which had been vaccinated as lambs the previous summer formed Group 2. Group1 were 30 age-matched, not previously vaccinated controls randomly selected from the same flock. All sheep grazed together throughout the trial.

l. Blinding: Laboratory Personnel performing faecal egg counts and haemoglobin analysis were blinded as to treatment groups.

24. Investigational and Control Products

s. Investigational Veterinary Product:

Name:	BarberVax	Batch No.:	V1. HCD220311D-005 V2. HCD220311C-006 V3. HCD220311C V4. HCD220311D-005
Composition:	Haemonchus antigen and saponin adjuvant	Expiry Date:	October , 2013
Dose Rate:	5ug antigen and 1mg saponin	WHP:	12 months

t. Source:
WormVax Laboratory
Animal Health Laboratory
Dept of Agriculture and Food Western Australia
444, Albany Highway
Albany, W.A 6330

u. Storage: Refrigerated 2 – 8^{o}C

v. Safety: A MSDS was not provided by the Sponsor. The IVP was administered using a specially designed safety vaccine gun to protect against accidental injection.

w. Assays: A Certificate of Analysis was not provided for the IVP.

x. Drug Disposal: All remaining IVP was retained at VHR pending disposal advice from the Sponsor.

25. Treatment

d. Dose Calculation: 1 mL IVP for each Group 2 animal; anthelmintic salvage drenches was calculated using Day 0, 77 and 161 bodyweights, or according to estimated bodyweights visually assessed by experienced VHR personnel when required.

e. Dose Preparation: The IVP was supplied ready for use and was mixed gently prior to use. Anthelmintic products were shaken prior to use.

f. Method of Dose Administration: Study animals were dosed according to the treatment regime detailed in Table 1 below.

Table 1 – Treatment Regime

Tx. Grp.	IVP Details	Dose Volume	Route	Tx Day(s)	No. Anim.
1	Negative controls	Various	Oral	As required for salvage- Days 0, 28, 42, 91, 105, 119, 133, 147, 161	Various (see individual treatment sheets)
2	IVP	1ml	Subcutaneous	Days 0, 28, 49, 91 and 133	30

The IVP was administered using a multi-dose injection gun, subcutaneously to the left hand side of the neck.

Anthelmintics were administered orally using either plastic disposable syringes or a generic multi-dose handpiece.

Study animals were observed at 2 hours post-treatment, no abnormalities were observed.

26. Schedule of events

Table 2 – Schedule of Events

Date	Day	Activity	Comments
29-Nov-12	0	Drafted all female sheep from MIHO2708, allocated to groups 1 and 2 . Balance required made up with MC. Weighed all trial animals, Collected blood from Gps 1+2, performed Hb analysis. Collected faeces from Gps 1and 2 (Counted and cultured). Vaccinated Gp2 animals, harvested plasma	Gp 3 not drenched because their mob had been drenched a few days prior, #32 treated on Hb analysis
13-Dec-12	14	Collected blood from Gp 2. Faeces from Gp 1, 2, (counted, cultured). Harvested plasma from Gp 2 bloods	
20-Dec-12	21	Collected blood for Hb analysis from Gps 1, 2. Faeces from Gp 1, 2, (counted, cultured). Harvested plasma from Gp 2 bloods	#14 treated for dag score, #2 treated for Hb
09-Jan-13	42	Collected blood for Hb analysis from Gps 1, 2. Faeces from Gp 1, 2 (counted, cultured), drenched Gp 3, treated Gp 2 with IVP. Harvested plasma from Gp 2 bloods.	

24-Jan-13	56	Collected blood for Hb analysis from Gps 1, 2. Faeces from Gp 1, 2 (counted, cultured). Harvested plasma from Gp 2 bloods	#74 treated on Hb
07-Feb-13	70	Collected blood for Hb analysis from Gps 1, 2. Faeces from Gp 1, 2 (counted, cultured). Harvested plasma from Gp 2 bloods	#20 treated for scour worms
21-Feb-13	84	Collected blood for Hb and plasma from Gp 1 and 2. Faeces from Groups 1,2 (counted cultured). Treated Gp 2 with IVP, Gp 3 drenched.	
07-Mar-13	98	Collected blood for Hb analysis from Gps 1, 2. Faeces from Gp 1, 2 (counted, cultured). Harvested plasma from Gp 2 bloods	#9,34 treated
21-Mar-13	112	Collected blood for Hb analysis from Gps 1, 2. Faeces from Gp 1, 2 (counted, cultured). Harvested plasma from Gp 2 bloods	#74 treated on for low Hb
05-Apr-13	126	Collected blood for Hb (Gp1 and 2) and plasma (Gp 2). Faeces from Groups 1,2 (counted cultured). Treated Gp 2 with IVP.	Treated #20 for scour worms
17-Apr-13	139	Collected blood for Hb analysis from Gps 1, 2. Faeces from Gp 1, 2 (counted, cultured). Harvested plasma from Gp 2 bloods	Treated #20 and #72 for scour worms
02-May-13	154	Collected blood for Hb analysis from Gps 1, 2. Faeces from Gp 1, 2 (counted, cultured). Harvested plasma from Gp 2 bloods	Treated #15 for scour worms
16-May-13	168	Weighed trial sheep. Collected blood for Hb (Gp1 and 2) and plasma (Gp 2). Faeces from Groups 1,2 (counted cultured). All trial animals treated with effective anthelmintic.	23 needed salvage drench for low Hb, not given as JC was drenching the whole mob.

*NAD- no abnormalities detected.

27. Test system

Species:	Sheep (Ovine)	**Number:**	Groups 1 and 2: 30 each Group 3: 15
Breed:	Merino	**Source:**	Commercial farm
Weight:	Groups 1 and 2: 11 – 20.4 kg	**Health and special requirements:**	Healthy animals. Not within existing WHP and ESI for animal health products used, as per National Vendor Declarations.
Sex:	Gps 1 and 2: Equal numbers of males and females	**Method of ID:**	Uniquely numbered visual ear tags
Age:	Gps 1 and 2: 2-6 months		

28. Animal management

f. Animal Welfare: Study animals were managed similarly and with due regard for their welfare. Animals were observed twice weekly for health problems according to AEC requirements. Animals were handled in compliance with UNE AEC no. 11/107 approved 01 Oct 11, and any applicable local regulations.

g. Health Management: All lambs in Groups 1 and 2 were vaccinated with 5-in-1 prior to study commencement.

h.

i. Housing: Routine management practices were followed. All trial animals were maintained as a single group in one paddock, with *ad-lib* access to pasture consisting of rye, clover and native grass species. Potable water was supplied *ad-lib*.

j. Animal Disposal: All animals were returned to the commercial herd on the source property at the conclusion of the study (with the exception of those deceased/missing animals detailed in Section 11b).

29. Study procedures

e. Trial Log: All scheduled and unscheduled events during the study were recorded.

f. Informed Consent: An "Owner Consent and Agreement" form was signed by the Owner and the Investigator prior to administration of treatment.

g. Weather Data: Data from the nearest Bureau of Meteorology weather station for the study period are included in the raw data.

h. Sample Storage, Transfer and Disposal: Sample storage, transfer and disposal were recorded on form STU-311/1 (Sample Storage Record). Replicate 1 and 2 samples were stored in separate temperature logged freezers at approximately -20^{o}C until dispatch. Replicate 1 plasma samples were dispatched for analysis on ice-bricks via same day dispatch. Replicate 2 plasma samples will be held in frozen storage at VHR facilities for a period of 12 months after the last sample collection timepoint, after which point they will be disposed of by high temperature incineration.

30. Assessment of effects

f. Body Weights: Animals were weighed at intervals outlined in section 9 - Schedule of Events and individual animal weights were recorded. Animal weigh scales were checked pre- and post-weighing with calibrated test weights. Body weights and body weight change during the study were compared between groups to determine treatment effects, if any, and are detailed in the results section of this report.

g. Blood Analysis: Single blood samples were collected from each animal in Groups 1 and 2 using 18 gauge needles into 8 mL lithium heparin vacutainers at intervals outlined in section 9 – Schedule of Events. Blood samples were processed for collection of plasma samples on the day of collection. Samples were individually labeled with the study no., animal no., study date and day, sample type. Frozen plasma samples were forwarded to Moredun Institute laboratories for anti-vaccine antibody titre analysis at completion of the study.

Key haematological parameters were compared to determine treatment effects, if any, and are detailed in the results section of this report.

h. Faecal Egg Counts / Larval Differentiation: Faecal samples were collected at intervals outlined in section 9 – Schedule of Events. Faecal samples were individually labeled with the animal ID. Individual faecal egg counts and group bulk larval differentiation were performed. Faecal egg counts and larval differentiation were compared to determine treatment effects, if any, and are detailed in the results section of this report.

Note: where an animal received a precautionary drench at any point throughout the study, the FEC sample collected within 14 days of the salvage drench was excluded from group mean FEC calculations. This rule did not apply to mass group drenches, or to drenching with ivermectin (partially ineffective against Haemonchus

i. Haemoglobin: Haemoglobin analysis results were compared between Groups 1 and 2 to determine statistical difference, if any, between treatment groups. Haemoglobin data are presented in Appendix 3 (tabulated data) of the study report. Haemoglobin was analysed using the Hemocue 201 Hb Analyser.

31. Statistical analysis

Faecal egg counts, blood haemoglobin concentrations and bodyweights were compared between Groups 1 and 2 using the t test, whereas the number of salvage treatments was compared by Fisher's exact test.
Egg count data from individual lambs which had been given a salvage drench less than 3 weeks previously were discarded from the group mean calculations.

32. Quality assurance

Veterinary Health Research has an independent Quality Assurance Unit which reviewed all aspects of quality assurance relating to this study. The Protocol, Study Report and raw data were subject to quality assurance inspection.

33. Data records

i. Amendments and Deviations:

To be added

j. Notes to File:

To be added

k. Change of Study Personnel: Scott Miller replaced Jess McLeod as Study Investigator from January, 2013, when she left the company.

l. Raw Data: All original raw data pages have been identified with the study number, signed and dated by the person making the observation and by the person recording the information, and will be paginated prior to appending to the final Study Report.

m. Communication Log: The Investigator maintained copies of all correspondence relating to the study. These will be archived with the final Study Report.

n. Permits: The study was covered by APVMA small trial permit no. PER 7250

o. Confidentiality: Confidentiality of the raw data, Study Report and results of the study, plus any information received from the Sponsor, will be maintained during and after the study. Publication of material will remain at the sole discretion of the Sponsor.

p. Study Report: The original signed Study Report with raw data, Analytical Report and Statistical Report appended will be submitted to the Sponsor. A copy of the Study Report, plus appendices, will be archived at Veterinary Health Research Pty Ltd, Trevenna Road, Armidale, NSW, Australia, for a minimum of five years.

34. Results

Deaths and data handling

No sheep died during the trial and the dataset was almost complete, such that no individual sheep's data had to be discarded.

Haemonchus Egg Counts

The vaccine substantially reduced the *Haemonchus* egg counts of all the animals in Group 2 as shown in Figure 1 and Table 1 below. Two "non-responder" animals, defined as a vaccinate with a mean egg count greater than the lower 95% confidence limit of the control group, were detected.

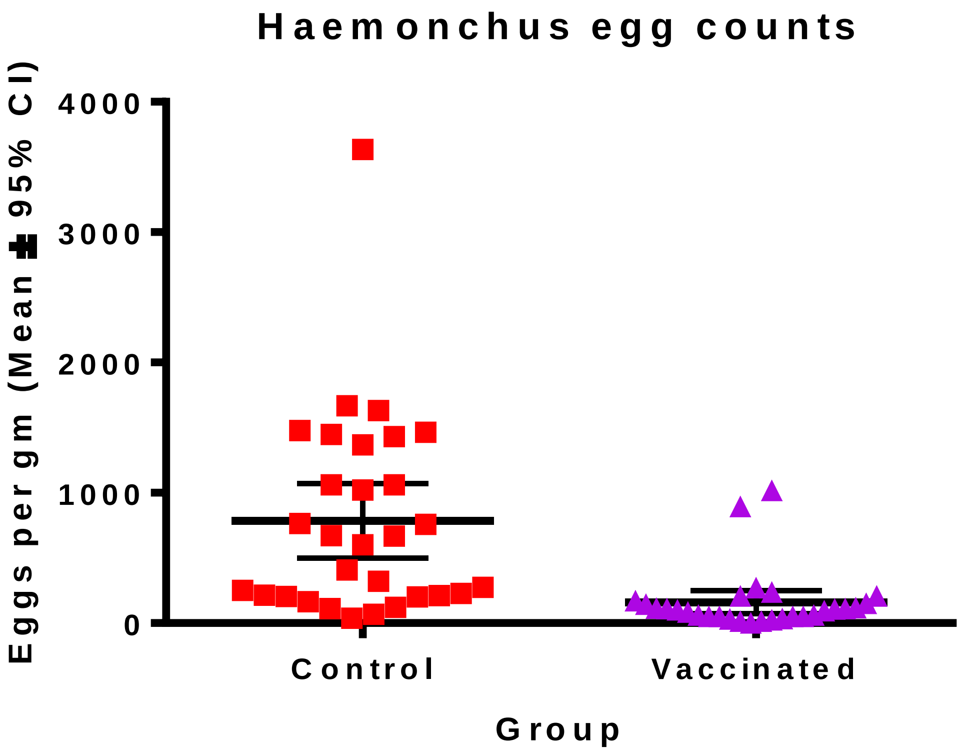


Figure 1. Overall effect of the vaccine on *Haemonchus* egg output between November 2012 and May 2013

Table 1. Group Arithmetic *Haemonchus* egg count means and vaccine efficacies.

	Mean over trial		
Day	**Control**	**Vaccine**	**%efficacy**
0	194	106	
14	44	4	90.7
21	149	2	98.9
41	820	48	94.1
56	1559	184	88.2
70	770	287	62.7
84	511	83	83.8
98	1343	487	63.8
112	2163	181	91.6
126	1076	438	59.3
140	133	44	66.8
153	370	11	97.0
167	557	53	90.4
mean	**745**	**148**	**82.2**

(the earliest the vaccine could have an effect is from day 14)

The scattergram in Fig 1 clearly shows that Barbervax substantially and significantly depressed the egg counts of the vaccinated sheep compared to the controls averaged over the duration of the trial. However, two vaccinated sheep failed to respond as judged by the fact that their counts exceeded the lower 95% confidence limit of the control counts (Fig 1).

Antibody status at the start of the trial.

At the start of this study ELISA titres in the unvaccinated sheep were very low and remained so throughout the trial (Table 2, Fig 2). In contrast, those of the vaccinated sheep were about 25 times greater at trial start and by two weeks after their first vaccination mean titres increased more than 4-fold (Table 2, Fig 2).

Table 2. Group mean antibody titres at the start of the trial

	Control day 0	Vaccinated day 0	Vaccinated day 14
mean	173	4409	13588
SE	100	787	3084

Relationships between the parameters studied over the course of the trial.

The first immunisation stimulated a secondary antibody response, as evidenced by the rapid increase in titre. Thereafter, each boost resulted in a spike of antibody production which tailed off until the next dose of Barbervax, although mean titres always remained at or above protective levels.

Mean egg counts in the control group showed a double peak profile, the first peak occurring in late January and the second in late March. The reason for the apparent trough during February and early March is not clear because the data in the table in Appendix 4 suggests that there was adequate rain for egg hatching and larval development.

Haemonchus was the most abundant larval genus found in the control coprocultures, but in the vaccinates *Trichostrongylus* almost always predominated (Table 9). Thus, although mean total egg output was nearly always lower in the vaccinates, the difference was even more obvious in the *Haemonchus* only egg count graph (Fig 2).

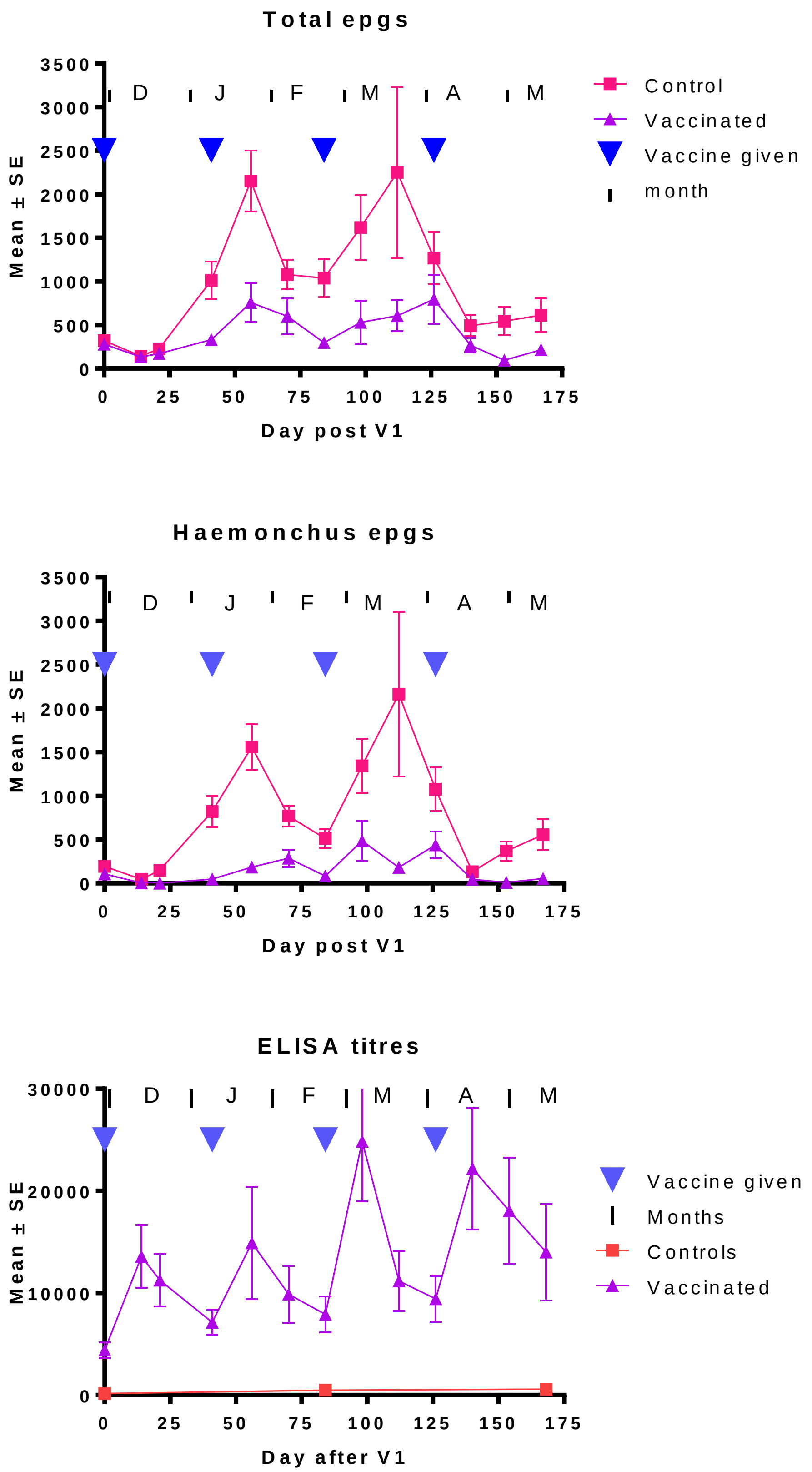
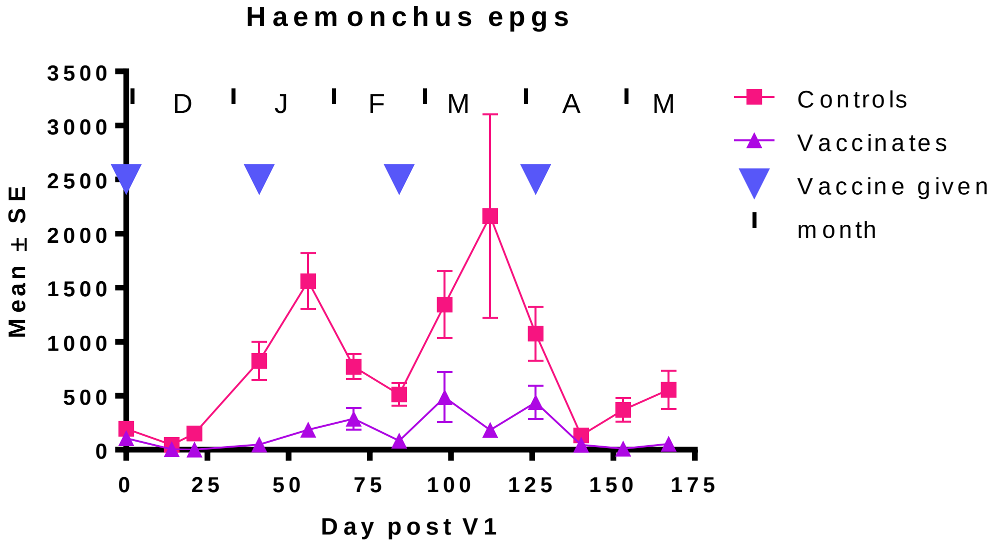


Figure 2. Kinetics of interventions, group mean total egg counts, *Haemonchus* specific egg counts and the anti vaccine antibody response.



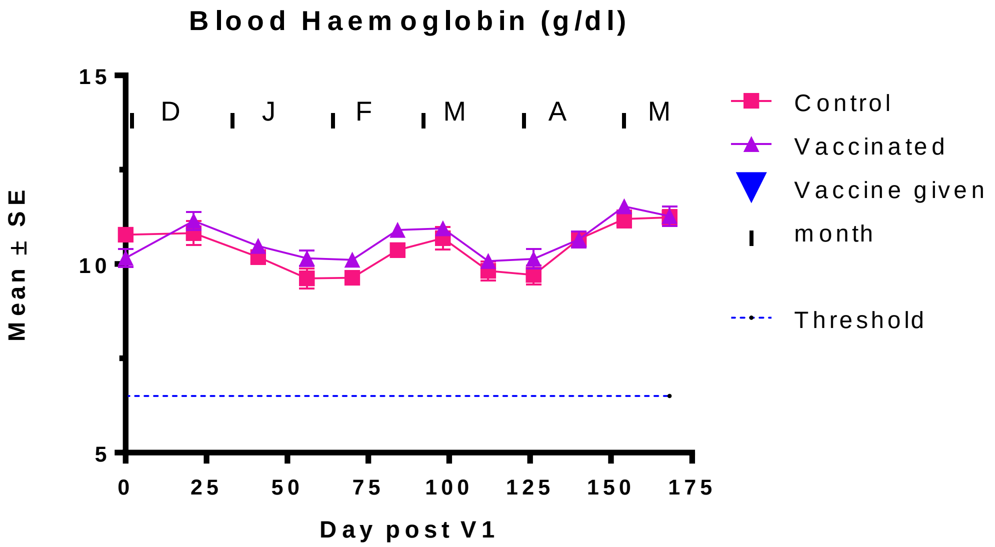


Figure 3. Kinetics of the *Haemonchus* specific egg counts in relation to blood haemoglobin concentrations.

Mean blood haemoglobin concentrations were very similar in both groups throughout the trial,

fluctuating between 9.5 and 11.5 g/dl which is within the normal range.

Three control sheep received a precautionary drench for anaemia, in each case almost certainly caused by *Haemonchus* because the accompanying egg counts were in the thousands. Three vaccinated sheep also had to be treated, but none of their associated egg counts were more than 100 epg, suggesting the cause was very unlikely to have been *Haemonchus*.

Two control and 3 vaccinated sheep were also drenched to remove scour worms.

Bodyweights

The lambs were weighed at intervals detailed in Section 9 of the Protocol. Group mean bodyweights in Kg are presented in the figure below. There was no significant difference in bodyweights ($p<0.05$; t-test) between vaccinated and control lambs at any time point of the study.

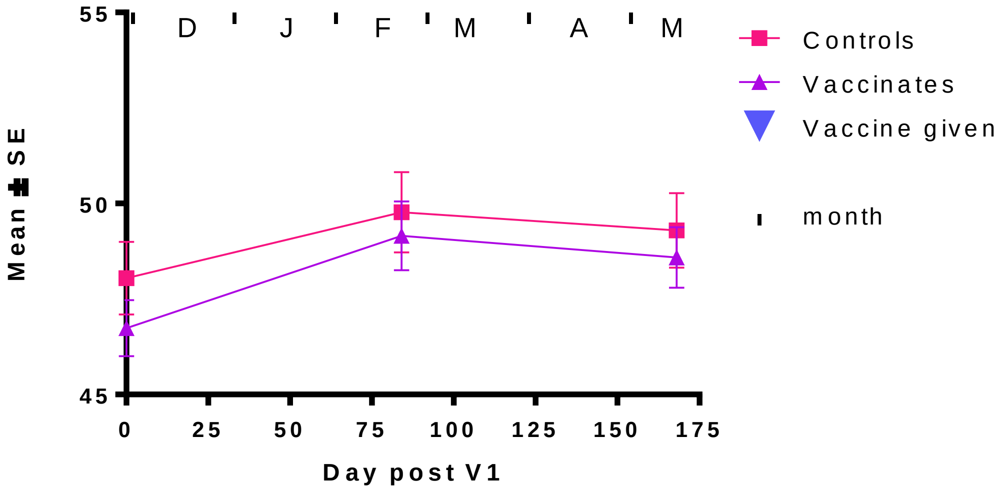


Figure 4. Yearling weights during the course of the trial

35. Discussion

It was very clear that the vaccine substantially reduced *Haemonchus* egg output over the course of the trial giving an overall efficacy of 82.2 percent.

Mean blood haemoglobin concentrations remained within the normal range in both groups throughout the trial and only 3 sheep, all controls, required a precautionary drench where the cause of anaemia was likely to have been Haemonchosis.

Four immunisations given 6 weeks apart were adequate to protect the vaccinated sheep, because all had been primed the previous season.

There were no other unscheduled events, health problems, adverse events, concurrent medications or mortality that were deemed treatment related.
There were no problems associated with administration of treatment with either the IVP or scheduled/salvage drenches.

APPENDIX 1

ABBREVIATIONS

VICH	International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Products
GCP	Good Clinical Practice
APVMA	Australian Pesticides and Veterinary Medicines Authority
MORAG	Veterinary Manual of Requirements and Guidelines
WAAVP	World Association for the Advancement of Veterinary Parasitology
VHR	Veterinary Health Research
SOP	Standard Operating Procedure
WHP	Withholding Period
ESI	Export Slaughter Interval
MSDS	Material Safety Data Sheet
ID	Identification
UNE	University of New England
AEC	Animal Ethics Committee
kg	Kilograms
g	Grams
µg	Micrograms
mg	Milligrams
mL	Millilitres
epg	Eggs per gram
Hb	Haemoglobin
g/dL	Grams per decilitre
FEC	Faecal Egg Count
NS	No Sample Collected
P/L	Proprietary Limited
UK	United Kingdom
^{o}C	Degrees Celsius
Tx	Treatment
Grp	Group
No.	Number
Anim.	Animals
NAD	No Abnormalities Detected
Hb	Haemoglobin
M (E) ovis	Mycoplasma (Erythrozoon) ovis

v1 – v5	Vaccination 1 – Vaccination 5
Haem.	*Haemonchus contortus*
Trich.	*Trichostrongylus*
Ost.	*Ostertagia*
Coop.	*Cooperia*
Oes.	*Oesophagostomum*

v1 – v5 Vaccination 1 – Vaccination 5
Haem. *Haemonchus contortus*
Trich. *Trichostrongylus*
Ost. *Ostertagia*
Coop. *Cooperia*
Oes. *Oesophagostomum*

APPENDIX 2

Standard Operating Procedures

SOP No.	SOP Title
SOP 6	Faecal egg counts
SOP 46	Collection of faeces
STU-308	Test and reference item receipt, storage, usage and disposal
STU-309	Study File Set-up
STU-311	Sample Identification, Storage, Transfer and Disposal
FLD-403	Identification of Study Animals
FLD-404	Feeding and Care of Study Animals
FLD-406	Weighing Study Animals (Livestock) and Weigh Scale Verification
FLD-408	Handling and Restraint of Study Animals
FLD-409	Health Monitoring of Study Animals
FLD-410	Chemical application
FLD-414	Blood Sample Collection
N/A	Enzyme-Linked Immunosorbent Assay (ELISA)

APPENDIX 3

Tabulated and Raw Data

Table 5. Tabulated Bodyweight Data

ID	Group	Bodyweights		
		Day 0	Day 84	Day 168
3	1	50	51.5	51
4	1	45	52	50.5
7	1	43.5	46	46
9	1	39	40	39.5
10	1	46	48	43
13	1	45.5	43	43.5
14	1	45	47	49
15	1	49.5	48	47
18	1	49	39	39
19	1	50	53.5	52
26	1	51	50.5	49.5
27	1	47.5	52	51
29	1	45	46	49
30	1	41.5	43	42.5
34	1	59.5	58.5	61
35	1	43.5	47.5	47.5
36	1	52.5	52	49.5
37	1	41.5	45	45.5
45	1	63.5	64.5	59
47	1	53	58.5	57.5
49	1	49	53.5	53.5
51	1	51	55	53
61	1	51	54	53
62	1	46.5	49.5	52
64	1	44.5	47	47.5
71	1	44.5	44.5	42.5
74	1	46.5	48.5	47.5
76	1	48	50.2	51.5
78	1	44.5	46.5	49
79	1	55	59	57
1	2	41.5	42	41
2	2	56	42.5	54.5
6	2	52	53	50.5
8	2	49.5	51	51.5
12	2	43	45	45
16	2	50	53.5	52.5
20	2	45	47	43.5
23	2	45.5	47	46
25	2	47	50	49.5
31	2	43	47.5	45
32	2	49.5	50.5	47.5

33	2	47.5	50.5	48
39	2	47	49.5	47
41	2	45.5	51	51
43	2	47	58.5	54
44	2	46	45.5	45.5
48	2	44.5	47.5	46.5
50	2	47	51	49
52	2	39	38.5	41.5
54	2	46.5	54	53
55	2	47	51.5	48.5
56	2	50	46.5	44
58	2	38	40	NS
59	2	55.5	59.5	55.5
60	2	46	50.5	52
66	2	47.5	48.5	49.5
67	2	52	53	52
68	2	44.5	45	42.5
72	2	45.5	48	46
80	2	44	57	57

Table 6. Tabulated Total FEC Data for yearling trial with precautionary treatments highlighted as follows:-

Drenched for scour worms

Drenched for Hb < 6.5 g/100ml

Animal ID	Group	Day	0	14	21	41	56	70	84	98	112	126	140	153	167
3	1		200	40	40	1240	1320	1880	720	1880	840	640	200	440	1160
4	1		680		200		2720	2080	2800	3160	3040	3840	1560	1320	1280
7	1		200	160	120	1800	3840	2160	1240	3560	840	4040	440	1840	
9	1		440	400	240	1360	4040	2440	3440	4880	2720	2320	1040	1080	0
10	1		720	120	680	2040	5480	1480	1320	2440	5080	960	520	400	4520
13	1		80	280	400	240		960	520	80	400	600	0	0	40
14	1		200	0	320		80	0	240	280	280	40	280	320	120
15	1		840	120	600	2720	1880	1560	1320	1200	5920	3600	3160	4480	
18	1		80	40	80	480	640	200	80	240	440	1120	280	320	80
19	1		600	40	160	1680	1720	960	2240	1680	3520	1200	760	1240	1560
26	1			280		1720	2200	920	2600	1320	1360	1120	1320	1200	2320
27	1		680		160	240	1400	520	720	1360	3320	7800	1200	400	2720
29	1		0	120	280	360	1200	1320		520		280	120	0	0
30	1		0	0	0	160	560	160	200	40	0	320	800	80	160
34	1		800	40	240	2720	5440	3640	4520	7360	120	0	40	40	120
35	1		0	0	80	360	400	80	0	0	0	120	0	0	0
36	1			560	120	1000	3960	1880	1040	640	0	1320	520	200	320
37	1		40	80	0	120	480	200	40	80	1600	240	40	0	0
45	1		240	0	80	0	240	640	120	240	200	840	200	160	0
47	1		160	0	0	120	960	480	40	80	520	720	320	80	120
49	1			0	0	480	0	0	0	0	40	0	0	40	0
51	1		360	0	0	640	3560	240	440	3840	1480	800	120	240	80

61	1	0	0	80	560	680	1400	1080	360	480	1120	240	480	320
62	1	0	40	0		1400	1600	120	80	1040	680	0	40	0
64	1	280	280	320	1080	1280	600	640	960	1120	1240	680	840	760
71	1	560	200	480	320	5680	760	1000	1320	760	1200	160	240	280
74	1	560	720	800	5320	6680		1080	7480	28480	0	80	0	120
76	1	480	280	880	960	2000	2880	2360	2600	1040	1520	560	720	1200
78	1		40	40	240	1560	760	40	240	160	200	80	120	0
79	1	80	160	160	360	960	560	80	640	440	160	40	0	40
1	2	0	120	120	440	560	320	640	520	280	200	120	0	320
2	2	160	40	280		0	160	400	760	440	600	280	520	1000
6	2		120	80	160	120	240	280	40	160	40	0	0	240
8	2	0		0	480	240	120	120	120	520	120	0	40	40
12	2	120	40	240	160	440	520	360	240	40	40	0	0	40
16	2	280	320	400	520	800	560	560	240	880	720	640	120	1200
20	2	1400	400	1120	520	6240	6320	0	1280	5120	7280	2440	40	640
23	2			0	40	0	280	0	40	480	200	80	240	280
25	2	400	80	320	360	600	560	440	880	560	1560	40	0	160
31	2	0	40	120	0	1480	1000	760	0	40	840	240		200
32	2	80		40	80	120	160	0	0	200	0	0	40	0
33	2	160	80	520		360	0	160	0	0	240	160	0	0
39	2	0	0	240	120	360	320	40	80	0	40	0	0	0
41	2	40	0	0	0		40	0	0	40	0	0	0	0
43	2	440	240	80	800	920	280	400	240	40	280	80	80	0
44	2	0	120	120	280	1160	480	360	200	160	440	120	40	80
48	2	200	0	200	1160	2080	880	480	760	1080	1120	640	360	600
50	2	600	240	480	1160	1400	640	480	80	400	80	80	160	160
52	2	80	40	80	0	400	320		280	440	640	200	240	200
54	2	120	40	0	160	160	40	200	0	0	0	120	0	
55	2	440	40	0		200	320	360	400	1920	2080	80	80	280
56	2		560	80	600	1040	600	440	1000	840	1440	440	160	0
58	2	720	120	0	640	560	120	200	440	960	400	320	320	160
59	2	680	240	0	280	760	1720	240	0	160	120	320	0	0
60	2	520	200	80	240	360	880	480	400	400	320	120	0	40
66	2		80	80	440		280	360	120	320	0	240	80	80

67	2	200	0		120	0	160	120	80	320	200	200	200	440
68	2	80	320	160	200	280	240	280	80	600	80	520	0	0
72	2		80	40	280	560	400	320	7600	1720	4800	520	0	0
80	2	240		40	40	0	0	80	0	0	0	0	0	40

Table 8. Tabulated Haemoglobin Data

Animal ID	Group	Day 0	Day 21	Day 41	Day 56	Day 70	Day 84	Day 98	Day 112	Day 126	Day 140	Day 154	Day 168
3	1	8.4	11.9	9.5	9.6	8.9	10.2	11.2	9.4	9.1	10.5	10.7	11
4	1	11.8	12.3	10.2	9.9	9.1	11.3	11.2	9.3	8.9	10.4	11.4	11.4
7	1	10.7	8.4	9.9	9.8	8.9	9.8	8.7	10.4	7.8	10	9.7	10.3
9	1	10.9	8.0	9.9	8.7	8.9	10.6	9.6	9.1	10.3	10.4	10.8	12.2
10	1	10.8	11.0	10.8	9.0	8.8	10.9	10.0	7.9	9.4	10	11.3	9.5
13	1	11.3	12.9	9.8	11.9	11.3	7.8	11.0	11.0	11.9	11	11.7	12.2
14	1	12.0	12.9	11.2	10.9	10.8	10.3	11.8	11.2	9.9	12.1	13.5	11.9
15	1	11.4	11.4	9.0	7.5	9.2	8.4	8.8	7.5	6.6	8.8	6.9	9.8
18	1	10.4	10.9	8.4	8.7	9.9	11.1	10.5	9.9	8.5	9.3	9.5	10.9
19	1	10.9	9.8	10.5	10.3	9.6	10.5	10.3	9.4	10.2	11.4	10.4	12.4
26	1	12.2	9.1	10.6	9.8	9.6	10.6	11.9	10.5	11.0	11.8	11.9	9
27	1	10.7	11.6	9.9	10.2	9.8	11.4	11.4	9.6	7.8	10	10.9	10.6
29	1	9.9	10.4	10.7	8.5	9.5	10.0	11.2	10.6	9.8	10.3	10.3	10.5
30	1	10.8	6.9	10.0	10.4	10.1	12.1	11.1	10.4	10.4	10.4	10.6	11.9
34	1	10.4	10.8	8.9	8.6	8.3	9.8	4.9	9.7	10.6	10.5	10.9	10.6
35	1	10.6	11.5	10.5	10.8	12.4	10.7	11.1	10.7	10.4	12.7	12	10.8
36	1	10.2	11.0	9.7	8.3	9.2	10.6	9.9	9.0	7.4	8.6	10.7	10.5
37	1	9.8	12.6	11.3	11.1	10.6	8.9	10.2	10.4	11.4	10.3	11.7	12.9
45	1	11.3	12.7	10.7	11.4	9.6	10.5	12.2	10.6	9.9	11.2	12.6	11.8
47	1	10.9	12.3	10.6	10.7	9.8	9.9	12.1	10.8	11.7	10.2	11.2	11.1
49	1	11.9	13.4	11.1	7.5	8.5	10.7	12.1	10.7	10.1	12.1	12.6	12.6
51	1	11.8	10.9	12.9	11.2	10.6	10.7	10.8	8.6	10.1	10.5	11.4	12.4
61	1	11.4	8.1	11.3	11.5	10.2	10.7	12.6	10.4	10.1	11.9	11.7	11.9
62	1	10.7	12.0	10.7	10.1	9.4	8.9	10.4	9.9	10.3	10.2	12	13.1
64	1	10.6	7.2	10.3	9.9	9.9	12.3	10.9	10.7	11.0	10.9	10.6	11.9
71	1	10.9	12.5	9.3	7.2	9.6	10.7	12.5	11.8	10.6	11.4	12.8	10.8
74	1	10.8	10.5	6.8	5.3	9.1	8.6	7.2	4.5	7.1	10.5	11.9	10.3
76	1	10.9	10.8	9.8	9.5	9.0	11.2	10.1	9.4	7.9	10.9	11.1	11.1
78	1	10.4	11.5	10.7	10.0	9.4	10.0	12.5	11.2	11.2	11.7	12.9	12.4

79	1	8.5	9.4	10.7	10.3	9.1	11.9	12.3	10.0	9.9	9.9	10.2	9.3
1	2	9.1	10.9	10.1	8.8	9.6	10.4	10.6	9.3	9.9	10.5	10.9	10.7
2	2	10.5	5.9	10.4	10.7	10.3	10.9	11.9	10.6	11.5	8.2	11.9	11.7
6	2	10.5	12.0	9.4	9.3	9.0	11.5	9.6	10.6	11.0	9.9	11.1	11.7
8	2	10.3	11.9	10.4	11.5	9.4	10.5	10.7	10.2	10.4	10.7	12.7	11.7
12	2	10.5	11.3	11.2	9.9	10.3	11.4	9.9	9.7	11.4	10.3	11	10.2
16	2	10.3	12.8	11.4	10.7	11.5	10.9	11.4	9.9	10.6	11	11.4	12.2
20	2	9.6	10.2	9.1	8.4	7.3	11.8	9.2	7.8	7.7	8.1	10.3	10.1
23	2	9.6	10.9	9.7	9.3	9.6	11.0	11.0	9.4	9.6	10.5	11.1	5.3
25	2	11.1	11.8	11.0	10.3	10.2	10.3	10.6	10.3	10.4	11.1	11.9	11.5
31	2	10.6	11.2	9.9	10.1	9.0	11.3	10.5	10.1	9.9	12.1	11.7	11
32	2	5.0	11.7	12.5	12.9	11.1	11.9	11.5	12.0	11.7	13.9	12.9	12.8
33	2	10.8	11.0	10.1	7.4	9.9	10.8	11.0	11.4	6.8	11.5	12.2	10.8
39	2	10.4	8.0	9.8	11.3	9.9	12.1	11.3	9.6	10.8	11.9	11.4	11.2
41	2	11.6	11.1	10.8	10.7	10.6	11.2	10.1	9.9	10.8	11.2	10.6	11.8
43	2	9.9	10.4	10.3	9.2	10.5	10.3	10.8	10.6	11.8	9.6	11.3	11
44	2	8.3	11.8	10.9	10.9	9.8	10.4	10.8	10.5	11.5	11.1	11.7	11
48	2	11.1	12.3	10.9	11.3	10.6	10.3	12.3	11.7	10.4	12	12.4	12.2
50	2	9.5	11.6	10.2	9.8	9.6	10.6	10.6	9.7	6.8	10.4	10.8	10.4
52	2	11.6	12.5	10.8	10.3	11.1	10.4	11.7	9.6	7.7	10	11.7	11.1
54	2	9.9	11.2	10.6	10.2	9.9	11.2	11.6	9.7	10.3	10.1	11.2	10.5
55	2	11.2	12.1	11.5	10.8	10.4	11.5	12.0	9.9	10.0	11.8	12.9	13.8
56	2	10.7	10.9	9.6	9.2	9.6	8.8	11.2	9.5	10.2	9.8	11.9	12.3
58	2	10.3	11.6	10.8	9.4	9.7	12.8	10.0	9.7	9.8	10.2	10.5	11.1
59	2	10.5	12.1	11.3	10.0	10.5	11.9	11.8	10.0	11.2	10.5	12.3	11.8
60	2	9.9	11.4	11.1	10.8	10.4	9.1	11.2	10.1	11.1	11.9	12.4	13.1
66	2	9.0	10.5	10.4	10.0	10.4	10.6	11.2	10.0	11.4	10.3	11	11.5
67	2	10.5	10.5	10.5	9.3	9.3	9.0	8.7	8.7	9.6	10.2	10.4	10.3
68	2	12.2	12.0	9.7	11.2	11.5	11.1	12.7	11.7	11.2	10.8	12.1	11.7
72	2	10.4	10.8	9.7	9.2	10.1	10.4	9.7	8.7	7.7	9.9	11.2	12.9
80	2	10.0	12.0	10.3	11.7	12.2	12.6	12.8	11.4	10.8	10.1	11	10.8

Table 9. Tabulated Larval Differentiation

Larval Differentiation

Group	Day	Treatment	Haem	Trich	Ost	Coop	Oes	TOTAL
1	0	Neg. controls	61	19	20	0	0	100
2		Vaccine	38	40	15	1	6	100
1	14	Neg. controls	31	50	13	1	5	100
2		Vaccine	3	49	47	0	1	100
1	21	Neg. controls	66	16	14	1	3	100
2		Vaccine	1	51	30	0	18	100
1	42	Neg. controls	84	12	3	0	1	100
2		Vaccine	15	72	6	1	6	100
1	56	Neg. controls	75	24	0	0	1	100
2		Vaccine	26	54	7	0	13	100
1	70	Neg. controls	69	19	3	0	9	100
2		Vaccine	48	13	22	1	16	100
1	84	Neg. controls	51	44	0	0	5	100
2		Vaccine	28	48	1	0	23	100
1	98	Neg. controls	83	12	0	0	5	100
2		Vaccine	92	7	1	0	0	100
1	112	Neg. controls	93	4	0	0	3	100
2		Vaccine	30	36	8	26	0	100
1	126	Neg. controls	82	18	0	0	0	100
2		Vaccine	55	30	7	0	8	100
1	139	Neg. controls	27	64	0	0	9	100
2		Vaccine	23	48	3	0	26	100
1	154	Neg. controls	68	32	0	0	0	100
2		Vaccine	11	80	5	0	4	100
3		Anthelmintic	0	0	0	0	0	0
1	168	Neg. controls	90	10	0	0	0	100
2		Vaccine	25	53	21	0	1	100
3		Anthelmintic	9	1	1	0	0	11

Table 10 Tabulated ELISA titres

APPENDIX 4

WEATHER DATA

Mean monthly rainfall (mm) at Deepwater post office, NSW during summer 2012-2013 (from the Australian Bureau of Meteorlogy)

Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
74.1	83.6	99.9	216.8	118.4	53.4	51.2	29.6

9.5 Appendix 5. VHR report of yearling vaccine trial conducted near Kingstown, NSW.

VETERINARY HEALTH RESEARCH PTY LTD

STUDY REPORT

Study Title: A field study to test the efficacy of a *Haemonchus* vaccine in yearling sheep during times of high parasite challenge. West New England district NSW, Australia.

Study No.: **MIHO 2895** **Sponsor Study No.:** N/A

Version No.: **2** **Version Date:** 4 November 2013

Author: Henry Chambers

Sponsor:	Name: Address:	Julie Fitzpatrick Moredun Group Director Moredun Institute The Moredun Group Pentlands Science Park Bush Loan Penicuik Midlothian Scotland, UK
Sponsor Monitor and Representative:	Name: Address: Phone: E-mail:	David Smith The Moredun Group Pentlands Science Park Bush Loan Penicuik Midlothian Scotland, UK +44 (0)131 445 6131 David.Smith@moredun.ac.uk
Investigator:	Name: Quals: Address:	Henry Chambers B. AgEc Grad.Cert. Rural Sci. Veterinary Health Research Pty. Ltd. Trevenna Road, Armidale, NSW 2350

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STUDY INVESTIGATOR

Compliance Statement

I, the undersigned, hereby declare that the report is a complete, true and accurate representation of the study and its results.

This study was conducted in accordance with the approved Protocol and with VHR Standard Operating Procedures (see Appendix 3), unless otherwise stated, and the study objectives were achieved. The study was conducted in compliance with:

- VICH GL9 Good Clinical Practice (June 2000)

There were no deviations from Protocol or any other circumstances considered to have affected the outcome of the study.

Signed: ____
Henry Chambers B.AgEc, Grad. Cert Rural Sci
Study Investigator

Date: ____

QUALITY ASSURANCE STATEMENT

Inspections were made by the Quality Assurance Unit of the various phases of the study described in this report. The date inspections were carried out and reported to the Investigator and to facility management are given below:

Inspection Date	Inspection Type	Phase Inspected	QA Auditor	Inspection Report issued

This report has been audited by the Quality Assurance Unit and is considered to be an accurate description of the methods and procedures used during the conduct of the study, and an accurate reflection of the raw data.

Signed: ______________________________

Leonora J Pearson DipRQA
Quality Assurance Manager

Date: ______________________________

1. Objective

To confirm the field efficacy of a *Haemonchus* vaccine in 12-14 month old sheep previously vaccinated and unvaccinated hogget sheep against *Haemonchus contortus* in the New England region of New South Wales, Australia. Data from this study may be used to support product registration.

2. Justification

Internal parasites of sheep are usually controlled by the strategic use of anthelmintic drugs. In the warmer, wetter parts of Australia *Haemonchus contortus* usually dominates the species of gut nematode parasites found in sheep. *Haemonchus* resistant to many of the commonly used anthelmintics are widespread in Australia and many other parts of the world. A vaccine to control *Haemonchus* would reduce the dependence on anthelmintics and be of great benefit to sheep producers.

An initial field trial on this property untilizing weaner sheep less than 12 months of age has shown that the vaccine in question is effective in reducing anthelmintic treatments and clinical haemonchosis. This trial is to utilize these previously vaccinated animals as 12-14 month old hoggets to determine efficacy in this age group, as well as determine what effect vaccination may have on the subsequent periparturient faecal egg count rise following joining.

3. Compliance

The study complied with the following national and international standards:

VICH GL9 Good Clinical Practice (issued June 2000)

4. Test sites

Animal Phase:
"Woodlands"
Torryburn Rd
URALLA
NSW 2358

Laboratory Phase:
Veterinary Health Research P/L
Colin Blumer Animal Health Laboratory
Trevenna Road
Armidale NSW 2350 Australia

Analytical Phase:
Moredun Institute
Pentlands Science Park
Bush Loan
Penicuik, Midlothian EH26 0PZ
SCOTLAND, UK

5. Study dates

Start date (animal phase): 27 Nov 2012
Finish date (animal phase): 14 May 2013
Finish date (laboratory phase): 11 October 2013

6. Study design

Thirty (30) 12-14 month old hogget sheep which had been vaccinated with Barbervax during the 2011-2012 summer formed the Vaccine group for this study. The Control group contained 30 age matched sheep randomly selected from the same flock, after excessively heavy or light "outliers" had been removed.

Sheep in Group 1 (30 animals) were the control group and consisted of unvaccinated animals and had blood and faecal samples collected on the days shown in **Table 2**. Sheep in Group 2 (30 animals) were treated with **IVP** and had blood and faecal samples collected on the days shown in **TABLE 2.** An additional fifteen (15) 12-14 month old sheep (hoggets), sourced from the same property, were selected and allocated to a third group – Group 3 as tracer animals (to indicate at intervals the level of H. contortus challenge) and had faecal samples collected and were treated with an effective anthelmintic on days shown in **TABLE 2**. All seventy-five (75) experimental sheep were maintained as a single grazing unit.

a. Experimental Unit: The experimental unit was the individual animal.

b. Animal Model: This study used hogget sheep (lambs born in spring 2011) due to their previous inclusion in this vaccine programme. Group 3 tracer animals were 12-14 month old hoggets selected form the same cohort as the Group 1 and 2 study animals.

c. Organism/Agent: All animals were exposed to a natural infection of parasites including Haemonchus contortus.

d. Inclusion Criteria: Animals were selected for the study if they met the criteria outlined in section 10 below.

e. Exclusion and Removal Criteria: No animals were excluded or removed from the study.

f. Allocation: Thirty (30) 12-14 month old hogget sheep (with as many of the previous years vaccinated female lambs as are available), with the balance made up of vaccinated castrated male lambs, previously included within the vaccinated group in the 2011/12 vaccination study (MIHO2708), were selected using the existing ear tags and allocated to Group 2 – vaccinates. An additional thirty (30) 12-14 month old hogget sheep, which were not vaccinated last year, were selected from the same cohort as the vaccinates and included as Group 1. Similar sex distribution as Group 2 animals will be included in this unvaccinated Group 1.

Fifteen (15) animals were selected at random from the larger (unvaccinated) group as they appeared in the animal handling facilities and allocated to Group 3 (tracers). The method of allocation and randomisation will be described in the raw data and Study Report.

Group mean bodyweights at allocation were analysed for significant differences between groups using One-Way Analysis of Variance and a commercially available software package (GraphPad).

g. Blinding: Laboratory Personnel performing faecal egg counts and haemoglobin analysis were blinded as to treatment groups.

7. Investigational and Control Products

a. Investigational Veterinary Product:

Name:	BarberVax	Batch Nos.:	HCD220311A-004 HCD220311C-004 HCD220311A-005 HCD220311A-007
Composition:	*Haemonchus* antigen and saponin adjuvant	Expiry Date:	October 2013
Dose Rate:	5ug antigen and 1mg saponin	WHP:	12 months

b. Source: The IVP was provided in a ready to use form from the laboratory engaged by the Sponsor manufacture the vaccine. The laboratory was:

WormVax Laboratory
Animal Health Laboratory
Dept of Agriculture and Food Western Australia
444, Albany Highway
Albany, W.A 6330

c. Storage: The IVP was stored in a temperature monitored refrigerator on VHR premises between 2 and 8°C. On treatment days it was transported to the trial sites in an esky on frozen ice packs.

d. Safety: A MSDS was not provided by the Sponsor (see Deviation #1). The IVP was administered using a specially designed safety vaccine gun to protect against accidental injection.

e. Assays: A Certificate of Analysis was not provided for the IVP (see Deviation #1).

f. Drug Disposal: All remaining IVP was retained at VHR pending disposal advice from the Sponsor. Disposal of the IVP will be documented.

8. Treatment

a. Dose Calculation: 1 mL IVP for each Group 2 animal; doses of anthelmintic salvage drenches were calculated using Day 0 and 84 bodyweights.

b. Dose Preparation: The IVP was supplied ready for use and was gently shaken prior to use. Anthelmintic products were shaken prior to use.

c. Method of Dose Administration: Study animals were dosed according to the treatment regime detailed in Table 1 below.

Table 1 – Treatment Regime

Tx. Grp.	IVP Details	Dose Volume	Route	Tx Day(s)	No. Anim.
1	Negative controls (Various anthelminitics)	As per product label	Oral	As required	30
2	IVP	1ml	Subcutaneous	Days 0, 42, 84, and 126	30
3	Tracers	As per product label	Oral	Days 42, 84 and 140*	15

* See Deviation 4

The IVP was administered subcutaneously into the left hand side of the neck using a multi-dose injection gun (see Deviation #3)

Anthelmintics were administered orally using either plastic disposable syringes or a generic multi-dose handpiece.

Study animals were observed at 2 hours post-treatment, no abnormalities were observed.

9. Schedule of events

Table 2 – Schedule of Events

Date	Day	Activity
Pre- Trial	---	Obtained Animal Ethics Committee approval; Confirmed trial arrangements with Sponsor and Farmer that ran MIHO2708, a previous study that was run in western portion of the NSW New England.
27-Nov-12	0	Drafted all female sheep from trial MIHO2708 and allocated to Groups 1 and 2 based on pre-existing eartags. Balance required in each group was made up with castrated males the same age from the same cohort. Selected and identified a further 15 hoggets for Group 3. Weighed all trial animals using verified electronic scales. Collected blood from Gps 1+2, performed Hb analysis. Processed blood samples, collected plasma and stored plasma frozen in 2 replicates. Collected faecal samples from Gps 1, 2 and 3 for FECs and Group larval differentiation. Vaccinated Group 2 with IVP.
11-Dec-12	14	Collected blood samples from animals in Group 2 and faecal samples from Gp 1,2 and 3 for FECs and group larval differentiation. Processed blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0C}
18-Dec-12	21	Collected blood samples from animals in Groups 1 and 2 for haemoglobin analysis, and faecal samples from Gp 1,2 and 3 for FECs and group larval differentiation. Processed Group 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0C}

08-Jan-13	42	Collected blood samples from animals in Groups 1 and 2 for haemoglobin analysis, and faecal samples from Gp 1,2 and 3 for FECs and group larval differentiation. Processed Group 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0C} Treated Gp 2 with IVP. Treated Group 3 with an effective anthelmintic (See Amendment 2)
22-Jan-13	56	Collected blood samples from animals in Groups 1 and 2 for haemoglobin analysis, and faecal samples from Gp 1,2 and 3 for FECs and group larval differentiation. Processed Group 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0C}
05-Feb-13	70	Collected blood samples from animals in Groups 1 and 2 for haemoglobin analysis, and faecal samples from Gp 1,2 and 3 for FECs and group larval differentiation. Processed Group 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0C}
19-Feb-13	84	Collected blood samples from animals in Groups 1 and 2 for haemoglobin analysis, and faecal samples from Gp 1,2 and 3 for FECs and group larval differentiation. Processed blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0C} Treated Gp 2 with IVP. Treated Group 3 with an effective anthelmintic (See Amendment 2). Weighed all trial animals present.
05-Mar-13	98	Collected blood samples from animals in Groups 1 and 2 for haemoglobin analysis, and faecal samples from Gp 1,2 and 3 for FECs and group larval differentiation. Processed Group 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0C}
19-Mar-13	112	Collected blood samples from animals in Groups 1 and 2 for haemoglobin analysis, and faecal samples from Gp 1,2 and 3 for FECs and group larval differentiation. Processed Group 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0C}
02-Apr-13	126	Collected blood samples from animals in Groups 1 and 2 for haemoglobin analysis, and faecal samples from Gp 1,2 and 3 for FECs and group larval differentiation. Processed Group 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0C} Treated Gp 2 with IVP.
16-Apr-13	140	Collected blood samples from animals in Groups 1 and 2 for haemoglobin analysis, and faecal samples from Gp 1,2 and 3 for FECs and group larval differentiation. Processed Group 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20 ^{o}C. Group 3 drenched with an effective anthelmintic.
30-Apr-13	154	Collected blood samples from animals in Groups 1 and 2 for haemoglobin analysis, and faecal samples from Gp 1,2 and 3 for FECs and group larval differentiation. Processed Group 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0C} Treated Gp 2 with IVP.
14-May-13	168	Collected blood samples from animals in Groups 1 and 2 for haemoglobin analysis, and faecal samples from Gp 1,2 and 3 for FECs and group larval differentiation. Processed blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0}C. Treated trial animals with an effective anthelmintic.

10. Test system

Species:	Sheep (Ovine)	**Number:**	Group 1: 30 Group 2: 30 Group 3: 15
Breed:	Merino		
Weight:	25-43.5kg	**Source:**	Commercial farm
Sex:	Gp 1- 16 female,14 MC Gp 2- 17 female,13 MC Gp 3- 2 female, 13 MC	**Health and special requirements:**	Healthy animals previously used in MIHO2708.
Age:	12 months	**Method of ID:**	Uniquely numbered visual ear tags

11. Animal management

a. Animal Welfare: All study animals were managed similarly with due regard to their welfare. Animals were observed for health problems according to AEC requirements. Animals were handled in compliance with UNE AEC no. 12/102 approved 01 November 12, and any applicable local regulations.

b. Concurrent Medications /Salvage Drenches : Any health problems or adverse events occurring during the study were recorded. Salvage drenches that were administered on the basis of a low haemoglobin content or high scour worm burden were recorded in the raw data. A summary is provided below.

#24, Group 2, was treated on Day 0 for low blood haemoglobin content.

#55, Group 1, was treated on Day 21 for low blood haemoglobin content.

#17, Group 1, and #13, Group 2, were treated on Day 56 for low blood haemoglobin content. #13 had been clipped for flystrike at some stage between Days 42 and 56.

#32, Group 1, was treated on Day 70 for low blood haemoglobin content.

#35,#42,#70,#78, (Group 1) and #71 (Group 2) were treated on Day 98 for a high proportion of scour worm according to individual faecal egg counts and larval differentials from the previous sampling point.

#7 and #71, (Group 2) were treated on Day 112 for low Hb (#7) and excessive scour worms (#71, going by the D98 FEC and larval differential).

#17 and #43 (Group 1) were treated on D126 for low blood haemoglobin content.

#32, #43 and #60 were treated on Day 140 for low blood haemoglobin content.

c. Health Management: All lambs in Groups 1 and 2 were vaccinated with 5-in-1 prior to study commencement.

d. Housing: Routine management practices were followed. All trial animals were maintained as a single group in one paddock, with *ad-lib* access to pasture consisting of native and improved pastures. Potable water was supplied *ad-lib* through plastic water troughs

e. Animal Disposal: All animals were returned to the commercial herd on the source property at the conclusion of the study.

12. Study procedures

a. Trial Log: All scheduled and unscheduled events during the study were recorded.

b. Informed Consent: An "Owner Consent and Agreement" form was signed by the Owner and the Investigator prior to administration of treatment.

c. Weather Data: Data from the nearest Bureau of Meteorology weather station for the study period are included in the raw data.

d. Sample Storage, Transfer and Disposal: Sample storage, transfer and disposal were recorded. Replicate 1 plasma samples were dispatched to Moredun Institute for analysis via WORLD COURIER on 24 June 2013. Replicate 2 plasma samples were held in frozen storage at VHR facilities for a period of 12 months after the last sample collection timepoint, after which point they will be disposed of by high temperature incineration.

13. Assessment of effects

a. Body Weights: Animals were weighed at intervals outlined in section 9 - Schedule of Events and individual animal weights were recorded. Animal weigh scales were checked pre- and post-weighing with calibrated test weights. Body weights were compared between groups to determine treatment effects, if any, and are detailed in the results section of this report.

b. Blood Analysis: Single blood samples were collected from each animal in Groups 1 and 2 using 18 gauge needles into 8 mL lithium heparin vacutainers at intervals outlined in section 9 – Schedule of Events. Blood samples were assayed for haemoglobin concentration and plasma was separated on the day of collection. Samples were individually labelled with the study no., animal no., study date and day, sample type. Frozen plasma samples were forwarded to Moredun Research Institute for anti-vaccine antibody titre determination at completion of the study.

c. Faecal Egg Counts / Larval Differentiation: Faecal samples were collected at intervals outlined in section 9 – Schedule of Events. Faecal samples were individually labelled with the animal ID. Individual faecal egg counts and group bulk larval differentiation were performed. Faecal egg counts and larval differentiation were compared to determine treatment effects, if any, and are detailed in the results section of this report. Note to File #1 details how salvage drenches were dealt with in the analysis of the Faecal Egg Counts.

d. Haemoglobin: Haemoglobin concentrations were determined in Groups 1 and 2 to determine statistical difference, if any, between treatment groups. Haemoglobin data are presented in **Appendix 3 (tabulated data**) of the study report. Haemoglobin was assayed using the Hemocue 201 Hb Analyser.

14. Statistical analysis

Faecal egg counts, blood haemoglobin concentrations and bodyweights were compared between Groups 1 and 2 using the t test, whereas the number of precautionary treatments was compared by Fisher's exact test.
Egg count data from individual lambs which had been given a precautionary drench less than 3 weeks previously were discarded from the group mean calculations.

15. Quality Assurance

Veterinary Health Research has an independent Quality Assurance Unit which reviewed all aspects of quality assurance relating to this study. The Protocol, Study Report and raw data were subject to quality assurance inspection.

16. Data records

a. Amendments and Deviations:

Amendment #1: The threshold for salvage drenching of study animals will be 6.5g/dL haemoglobin, instead of 8.5g/dL. Previous studies had demonstrated that 6.5g/dL is a more realistic threshold that prevents needless salvage drenching of a large number of animals.

Amendment #2: Group 3 (tracers, n=15) will be drenched with an effective anthelminitic every 6 weeks.

Deviation #1: The Sponsor did not provide an MSDS or Certificate of Analysis for the IVP. It wasn't deemed essential for pilot batches of the vaccine. This deviation had no effect on the outcome of the trial.

Deviation #2: The study title changed from: A field study to test the efficacy of a *Haemonchus* vaccine in previously vaccinated and unvaccinated 12-14 month old yearling sheep during times of high parasite challenge. North New England district NSW, Australia.
To
A field study to test the efficacy of a *Haemonchus* vaccine in previously vaccinated and unvaccinated 12-14 month old yearling sheep during times of high parasite challenge. West New England district NSW, Australia.

The change occurred because the trial site was in the western part of the New England Region. This deviation had no effect on the outcome of the trial.

Deviation #3: The IVP was administered using a multi dose vaccinator gun into the left hand side of the neck, after the wool had been parted to expose the skin, instead of using a syringe into the axilla region. This deviation had no effect on the outcome of the trial.
Deviation #4: Group 3 was not drenched on Day 0, as the mob that they were drawn out of had been drenched quite recently. In addition Group 3 was not drenched every 6 weeks as per Amendment 2. The drenches were scheduled to occur on Days 42, 84 and 126. Group 3 was drenched on Days 42, 84 and 140.

This deviation had minimal effect on the outcome of the trial as the data from Group 3 faecal egg counts still showed challenge during that period of the trial.

Deviation #5: The activities on Day 28 were rescheduled to Day 21 because Day 28 fell on Christmas Day. This deviation had no effect on the outcome of the trial.

Deviation #6: Group 2 was not drenched with oral Ivermec on Day 84 as faecal egg counts and larval differential results showed that it was not warranted. This deviation had no effect on the outcome of the trial.

Deviation #7: Some of the sheep used in the trial were still under the 12 month withhold from the previous trial, and Group 3 consisted of 13 male castrate and 2 female sheep, instead of all of one sex or equal numbers of sexes. This deviation had no effect on the outcome of the trial.

Deviation #8: 3 sheep were not administered a salvage drench after tripping the salvage drench threshold calculated by the method outlined in NTF 2. They were:

#71 - not drenched on Day 84 after the individual FEC tripped the threshold on Day 70. It was drenched on Day 98.

#37, 3 – individual FECs were greater than the calculated threshold after the Day 84 collection.

This deviation had minimal effect on the outcome of the trial.

Deviation #9: A number of sheep were missing at sampling points during the trial. The sheep missing at each sampling time are listed below:

Day 42 -#69, #48
Day 56 -#69
Day 70 - #6, #43, #69, #13
Day 84 - #23, #69
Day 98 - #23, #69
Day 112 - #23, #69
Day 126 - #23, #69
Day 140 – #13, #20, #23, #24, #29, #53, #61, #69, #77, #96, #95
Day 154 -#20, #23, #24, #29, #38, #41, #53, #61, #69, #77, #96, #95
Day 168 -#20, #23, #24, #29, #53, #54, #61, #69, #77, #96, #95

During January and February 2013 the trial site was subject to flash flooding several times. This flooding demolished fencing across waterways and allowed trial animals to roam into other paddocks.

In late April the fences were broken by bulls fighting through the fences, which allowed sheep to escape from their paddock.

This deviation may have had some effect on the outcome of the trial by reducing the number of animals available for statistical analysis.

b. Notes to File:

Note to File #1: Where an individual animal received a precautionary drench at any point throughout the study, the FEC result obtained within 14 days after the precautionary drench was excluded from group mean FEC calculations. This rule did not apply to mass group drenches, or to drenching with ivermectin (partially ineffective against *Haemonchus*).

Note to File #2: The study used formulation that had been received prior to the commencement of the study, and formulation that was received during the study.

Note to File #3: The threshold for calculating if a salvage drench was necessary was calculated by the calculating the proportion of larvae in the larval differential that wasn't *Haemonchus* spp,

Individual Drench Threshold= (1500/b)*100

Where b is the proportion of larvae in the Group larval differential that *wasn't Haemonchus* spp. and 1500 was the highest number of scour worm larvae allowed per sheep.

This was calculated for each group after the group larval differentials were counted (typically 10-12 days after collection) and acted on at the next sampling point if the individual sheep FEC was higher than the threshold.

The Group Drenching threshold was calculated in a similar manner except the highest number of allowable scour worm larvae was lowered to 1000 and the group mean was substituted for the individual sheep FEC.

c. Change of Study Personnel:

Change of Study Personnel #1: Jess McLeod replaced Bruce Chick as Study Investigator at commencement of study on 03 November, 2011, due to a workload re-structure.

Change of Study Personnel #2: Henry Chambers replaced Jess McLeod as Study Investigator on 23 January 2013, due to Jess McLeod resigning from Veterinary Health Research.

d. Raw Data: All original raw data pages have been identified with the study number, signed and dated by the person making the observation and by the person recording the information, and will be paginated prior to appending to the final Study Report.

e. Communication Log: The Investigator maintained copies of all correspondence relating to the study. These will be archived with the final Study Report.

f. Permits: The study was covered by APVMA small trial permit no. PER 7250

g. Confidentiality: Confidentiality of the raw data, Study Report and results of the study, plus any information received from the Sponsor, will be maintained during and after the study. Publication of material will remain at the sole discretion of the Sponsor.

h. Study Report: The original signed Study Report with raw data, Analytical Report and Statistical Report appended will be submitted to the Sponsor. A copy of the Study Report, plus appendices, will be archived at Veterinary Health Research Pty Ltd, Trevenna Road, Armidale, NSW, Australia, for a minimum of five years.

17. Results

a. Missing Trial Sheep and Data Handling: Where more than 3 of the samples collected over the course of the trial were missing from a sheep, all the data from that animal was discarded from the analysis. This happened with 5 controls and 4 vaccinates. See Deviation #9.

b. *Haemonchus* Egg Counts: The vaccine substantially reduced the *Haemonchus* egg counts of all the animals in Group 2 as shown in **Figure 1 and Table 1** below. One "non-responder" animal, (defined as a vaccinate with a mean egg count greater than the lower 95% confidence limit of the control group) was detected.

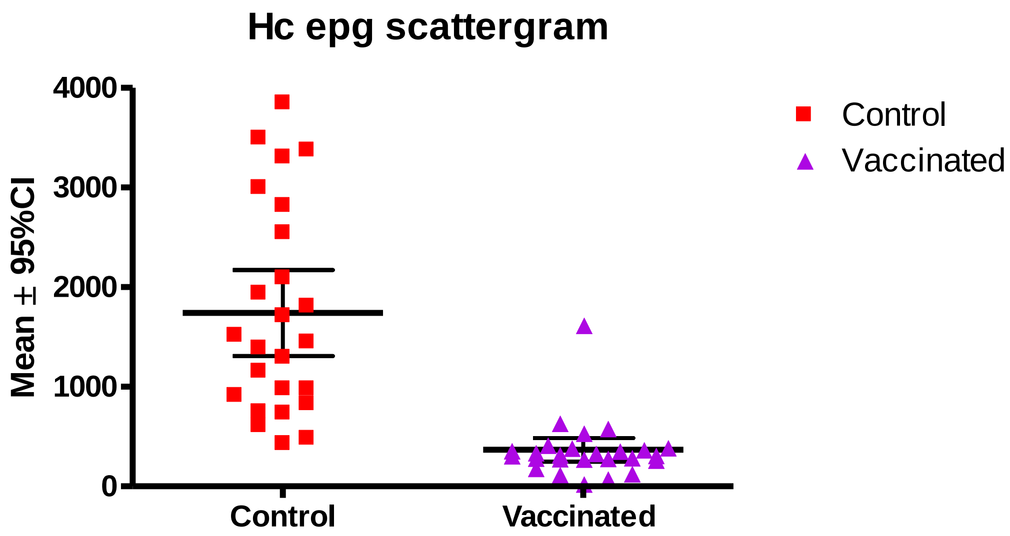

Figure 1 - Overall effect of the vaccine on *Haemonchus* egg output between November 2012 and April 2013.

Table 3- Group Arithmetic *Haemonchus* egg count means and vaccine efficacies.

Day	Group means		%
after V1	Control	Vaccine	Efficacy
0	65	38	*

14	192	125	35.0
21	445	154	65.3
42	1860	638	65.7
56	2282	440	80.7
70	3498	722	79.4
84	773	377	51.2
98	1902	247	87.0
112	1821	102	94.4
126	2430	289	88.1
140	3724	548	85.3
154	1826	637	65.1
168	1585	477	69.9
Mean	**1723**	**369**	**70.0**

* The earliest the vaccine could have an effect is from Day 14)

c. Antibody status at the start of the trial: At the start of this study ELISA titres in the unvaccinated sheep were very low and remained so throughout the trial (**Table 4, Fig 2**). In contrast, those of the vaccinated sheep were substantially greater at trial start and by two weeks after their first vaccination mean titres increased more than 4-fold (**Table 4, Fig 2**).

Table 4 - Group mean antibody titres at the start of the trial

	Control Day 0	Vaccinated Day 0	Vaccinated Day 14
mean	61	2,146	9,558
SE	23	263	2,352

d. Relationships between the parameters studied over the course of the trial: Within 14 days of their first immunisation, the mean antibody level in the vaccinated group attained a titre of greater than 9,500, clear evidence of a secondary response **(Fig 2).** Thereafter clear spikes in titre were apparent after each boost with group means always exceeding 4,000. Meanwhile control titres remained at negligible levels throughout.

Mean total egg counts in the control sheep built up rapidly during December and January to about 4,000 epg by the start of February (**Fig 2**), before dipping to around the 2,000 epg level until mid March. A second peak occurred by mid April before the counts tailed off in May. Total egg counts in the vaccinates were consistently lower, except at the final sampling in May **(Fig 2).**

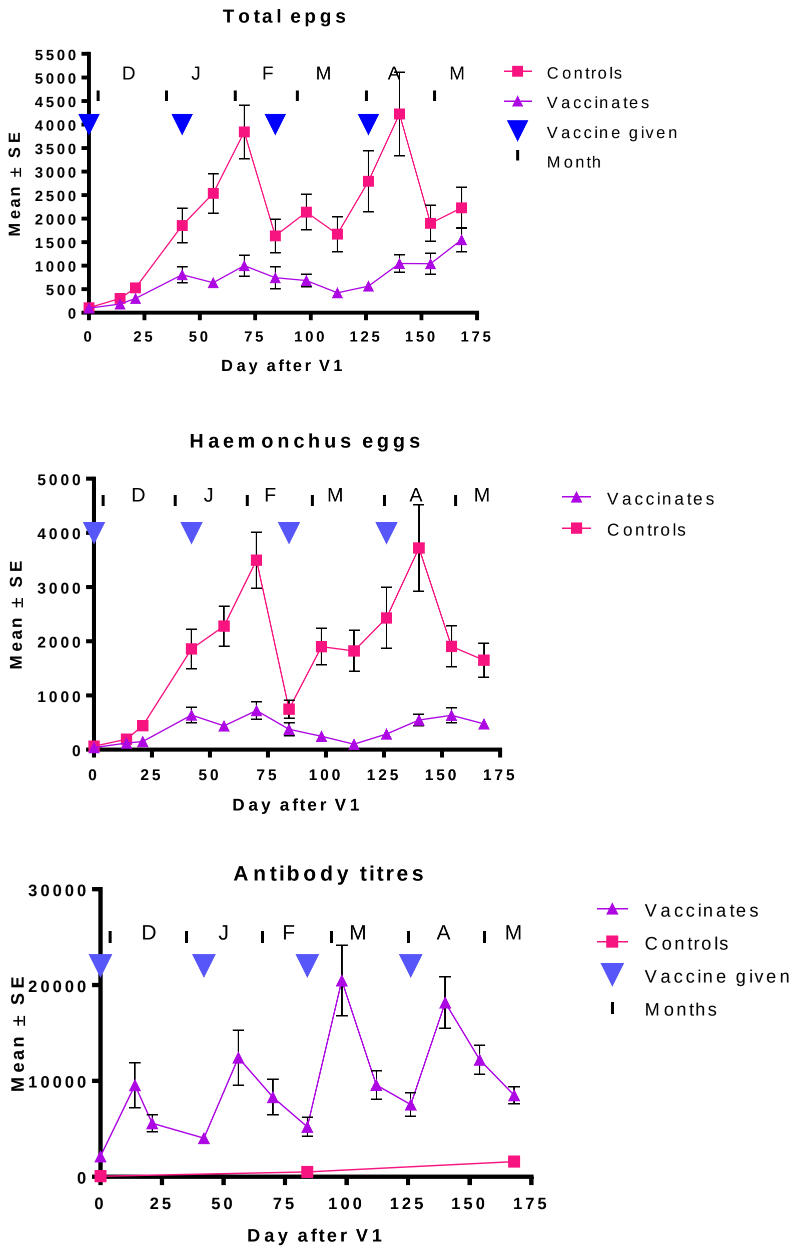

Figure 2 - Kinetics of interventions, group mean total egg counts, *Haemonchus* specific egg counts and the anti vaccine antibody response.

Haemonchus specific and total egg counts followed broadly similar trajectories in the control group, but there was a clearer difference between vaccinate and control *Haemonchus* counts especially towards the end of the trial when the proportion of *Trichostrongylus* eggs in the total counts increased (Fig 2, Table 9).

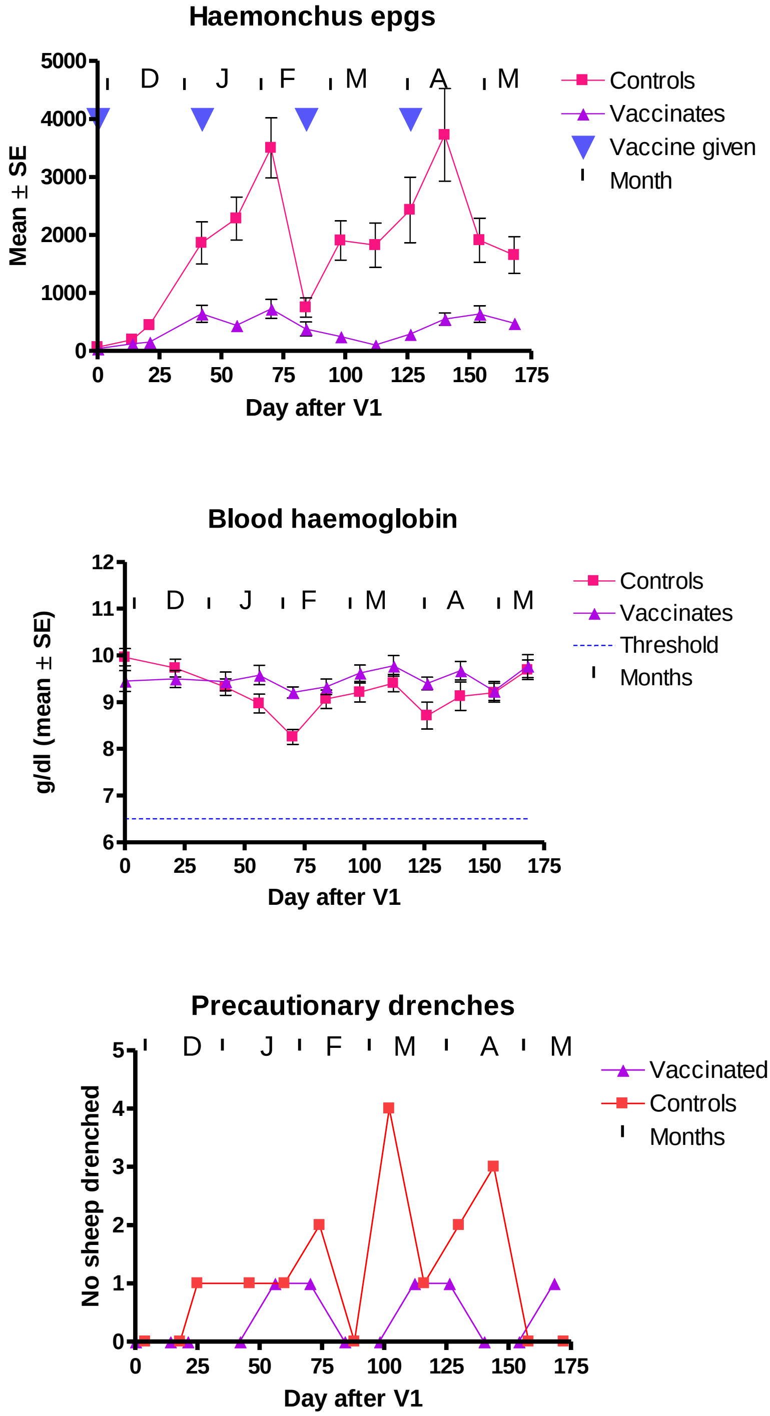

Figure 3 - Kinetics of the *Haemonchus* specific egg counts in relation to blood haemoglobin concentrations and to precautionary drenches.

Mean blood haemoglobin concentrations remained fairly steady in the vaccinated group during the trial at between 9 and 10 g/dl. During December control blood haemoglobin concentrations usually exceeded vaccinate values, but from January to mid April they were marginally lower, except in early February and early April when the difference between the groups was more marked.

Fifteen control sheep received precautionary drenches on Days 42, 84 and 140, three times the number given to the vaccinated sheep.

Significant diff ? Sometimes given mistakenly? See Table 6.

but in one case it was to deal with intestinal parasites and in the other the accompanying egg count was considered too low for the anaemia to have been caused by *Haemonchus*.

Table 5 - Number of precautionary drenches

Day	**No. drenched**		**Fishers exact test**
after V1	**Control**	**Vaccine**	
0	0	0	
14	0	0	
21	1	0	
42	1	0	
56	1	1	
70	2	1	
84	0	0	
98	4	0	
112	1	1	
126	2	1	
140	3	0	
154	0	0	
168	0	1	
Total	**15**	**5**	

e. Bodyweights: The lambs were weighed at intervals detailed in Section 9. Group mean bodyweights in Kg are presented in the figure below. There was no significant difference in bodyweights ($p<0.05$) between treated and untreated lambs at any time point of the study.

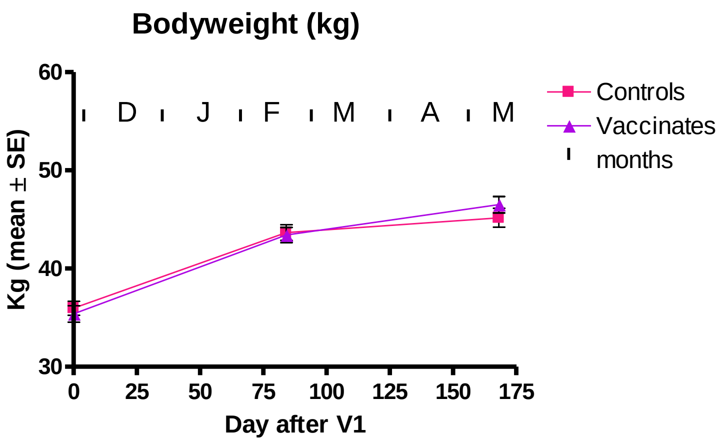

Figure 4 - Yearling weights during the course of the trial.

18. Discussion

Immunisation of the yearlings in December, some 8 months after they had received their last boost in March, resulted in an anamnestic antibody response, which was maintained at high levels for the duration of the trial by three further boosts.

These titres seemed to be adequate to greatly suppress *Haemonchus* egg production, reduce the ensuing degree anaemia (even though it was quite mild in this trial) and the consequent number of precautionary drenches.

The vaccinated sheep grew as quickly as the controls and no adverse signs attributable to the immunisation regime were recorded.

APPENDIX 1

ABBREVIATIONS

VICH	International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Products
GCP	Good Clinical Practice
APVMA	Australian Pesticides and Veterinary Medicines Authority
MORAG	Veterinary Manual of Requirements and Guidelines
WAAVP	World Association for the Advancement of Veterinary Parasitology
VHR	Veterinary Health Research
SOP	Standard Operating Procedure
WHP	Withholding Period
ESI	Export Slaughter Interval
MSDS	Material Safety Data Sheet
ID	Identification
UNE	University of New England
AEC	Animal Ethics Committee
kg	Kilograms
g	Grams
µg	Micrograms
mg	Milligrams
mL	Millilitres
epg	Eggs per gram
Hb	Haemoglobin
g/dL	Grams per decilitre
FEC	Faecal Egg Count
NS	No Sample Collected
P/L	Proprietary Limited
UK	United Kingdom
^{o}C	Degrees Celsius
Tx	Treatment
Grp	Group
No.	Number
Anim.	Animals
NAD	No Abnormalities Detected
Hb	Haemoglobin
M (E)	Mycoplasma (Erythrozoon) ovis

ovis

v1 – v5	Vaccination 1 – Vaccination 5
Haem.	*Haemonchus contortus*
Trich.	*Trichostrongylus*
Ost.	*Ostertagia*
Coop.	*Cooperia*
Oes.	*Oesophagostomum*
v1 – v5	Vaccination 1 – Vaccination 5
Haem.	*Haemonchus contortus*
Trich.	*Trichostrongylus*
Ost.	*Ostertagia*
Coop.	*Cooperia*
Oes.	*Oesophagostomum*
MC	Male Castrate

APPENDIX 2

STANDARD OPERATING PROCEDURES

SOP No.	SOP Title
SOP 6	Faecal egg counts
SOP 46	Collection of faeces
STU-308	Test and reference item receipt, storage, usage and disposal
STU-309	Study File Set-up
STU-311	Sample Identification, Storage, Transfer and Disposal
FLD-403	Identification of Study Animals
FLD-404	Feeding and Care of Study Animals
FLD-406	Weighing Study Animals (Livestock) and Weigh Scale Verification
FLD-408	Handling and Restraint of Study Animals
FLD-409	Health Monitoring of Study Animals
FLD-410	Chemical application
FLD-414	Blood Sample Collection

APPENDIX 3

TABULATED DATA

Table 6. Tabulated Bodyweight Data

Animal ID	Group	Bodyweights (kg)		
		Day 0	Day 84	Day 168
1	1	35.0	43.0	48.5
3	1	42.5	48.0	49.0
6	1	36.0	40.0	33.5
15	1	36.5	42.0	43.0
17	1	31.0	39.0	41.0
18	1	33.0	40.0	44.5
20	1	38.5	47.0	---
29	1	39.0	52.0	---
32	1	36.0	47.0	48.5
34	1	41.0	48.0	49.5
35	1	40.0	45.5	48.5
36	1	31.5	39.5	43.0
37	1	32.5	42.0	45.0
42	1	29.5	38.0	45.0
43	1	34.5	40.0	36.0
44	1	35.5	39.0	42.0
48	1	39.0	42.5	47.5
51	1	36.5	46.0	44.5
52	1	40.5	45.0	48.5
54	1	43.5	54.5	55.5
55	1	38.0	43.5	47.0
57	1	39.5	49.5	51.5
60	1	35.5	43.5	47.0
61	1	30.0	43.5	---
62	1	37.0	49.0	51.0
66	1	31.0	42.5	43.0
67	1	30.5	38.0	41.0
70	1	37.0	36.5	39.5
77	1	38.0	44.5	---
78	1	30.5	41.5	41.5
2	2	40.0	47.5	52.5
4	2	26.5	34.5	37.0
7	2	30.0	39.0	40.5
8	2	29.0	40.0	41.5
10	2	39.0	46.0	50.0
12	2	30.0	39.5	41.5

13	2	34.0	34.5	38.0
14	2	41.0	46.5	51.0
22	2	40.0	47.0	48.5
23	2	41.0	---	---
24	2	25.5	35.0	---
26	2	36.0	42.0	45.0
27	2	33.0	43.0	47.0
30	2	33.5	43.0	48.0
33	2	37.5	48.5	50.0
38	2	37.0	45.0	50.5
41	2	36.5	46.0	46.5
47	2	35.0	42.0	43.5
49	2	36.5	45.0	49.0
53	2	37.5	49.0	---
58	2	39.0	47.0	49.0
59	2	36.0	43.0	46.5
63	2	39.0	43.5	47.0
69	2	25.0	---	---
71	2	36.5	44.0	48.5
74	2	37.0	44.0	47.0
75	2	33.0	43.5	44.0
76	2	38.0	46.5	49.0
80	2	41.0	49.5	51.5
96	2	38.5	41.5	---

Table 7. Tabulated Total FEC Data.

Numbers in Bold Italics denote animal drenched for low blood haemoglobin, Numbers in bold denote animal drenched for scour worms. NS denotes no sample collected.

Animal ID	Group	0	14	21	42	56	70	84	98	112	126	140	154	168
		Eggs Per Gram												
1	1	0	120	120	560	2040	2120	2160	1800	1680	1520	3600	1360	720
3	1	80	600	880	4280	9320	7960	4440	4960	2440	1200	1360	1040	560
6	1	0	40	0	480	1640	NS	520	1000	160	560	2000	2080	1880
15	1	80	160	600	880	520	2800	200	1280	280	1280	1400	1800	3680
17	1	160	1400	1720	9640	***7560***	0	240	3160	8200	***11000***	0	0	40
18	1	0	40	160	640	560	1200	800	2960	2080	1720	4960	3880	2480
20	1	240	80	360	1000	1440	2520	560	1600	720	1800	NS	NS	NS
29	1	40	280	400	1360	1760	2880	280	960	320	1320	NS	NS	NS
32	1	520	760	760	4640	5320	***13400***	40	240	3000	7560	***14960***	0	80
34	1	80	0	240	760	880	1000	440	440	640	800	1080	440	480
35	1	240	360	800	3000	3320	6120	5920	**240**	0	0	80	520	1080
36	1	0	120	280	480	760	1920	520	5200	400	280	NS	1520	920
37	1	200	600	1320	2040	5480	6160	2880	720	840	720	3640	3800	4080
42	1	520	760	840	1280	3640	4840	8040	**9480**	0	440	6520	2800	6320
43	1	40	80	600	1720	1600	NS	2280	NS	5920	***13800***	0	0	0
44	1	0	160	200	1160	2320	4440	320	680	1640	680	1880	320	560
48	1	0	160	200	NS	2200	4120	360	840	NS	440	2520	1720	4320
51	1	80	240	840	1640	6120	4160	520	2160	1440	1400	1720	840	1400
52	1	0	160	400	2160	2920	2480	1800	1400	1560	3040	4880	2120	3200
54	1	40	0	120	640	280	1600	360	800	440	240	800	520	NS
55	1	80	NS	280	40	0	40	120	200	80	1000	2480	800	2200

57	1	NS	360	720	760	1760	4440	1560	1960	4440	7920	9080	6480	7480
60	1	200	160	600	2960	4400	2160	2560	4840	4840	6320	***16040***	NS	0
61	1	120	160	720	1920	1760	2360	1920	1880	1920	3280	NS	NS	NS
62	1	0	240	200	1160	680	3400	600	1000	1080	2600	5960	6440	5600
66	1	0	0	200	160	240	1920	400	1240	3280	5720	3640	4920	5400
67	1	200	680	1440	400	1160	800	240	1520	600	1800	1400	800	360
70	1	40	600	520	4800	4280	10280	4840	**4640**	0	0	7080	1200	1760
77	1	120	360	320	2280	1160	4720	720	1520	440	4360	NS	NS	NS
78	1	0	160	40	880	960	3960	3320	**3240**	0	0	80	240	1200
2	2	40	40	80	880	600	1120	880	480	480	840	640	160	240
4	2	0	80	240	800	240	600	440	880	1040	560	2600	720	1240
7	2	40	200	400	600	560	960	1680	2680	***1880***	0	40	960	760
8	2	80		240	840	240	280	960	760	200	600	800	600	640
10	2	80	120	80	200	360	320	160	320	120	280	280	280	640
12	2	0	0	80	0	200	40	120	400	40	120	120	280	800
13	2	40	280	480	NS	***1920***	NS	0	120	320	1160	NS	3560	2920
14	2	0	40	0	NS	40	80	80	80	120	80	40	80	40
22	2	160	520	800	920	360	800	360	520	280	520	400	480	1320
23	2	80	680	440	2440	1480	1240	NS	NS	NS	NS	NS	NS	NS
24	2	40	40	0	880	640	680	880	880	840	1000	NS	NS	NS
26	2	0	0	0	160	440	600	320	480	120	360	1760	880	3160
27	2	80	120	80	240	80	720	120	240	120	160	1120	320	1240
30	2	80	80	120	560	400	480	240	160	200	240	2640	840	440
33	2	320	320	280	920	600	80	200	600	360	1000	2000	1680	1760
38	2	400	NS	1040	320	600	960	640	1520	440	680	1640	NS	1400
41	2	40	280	240	1720	480	1120	880	560	960	880	80	NS	1520
47	2	0	0	80	160	1200	800	600	360	800	1000	520	360	800
49	2	80	80	160	1120	1080	1880	280	320	440	480	3040	2480	3480

53	2	120	240	760	720	1520	1120	720	440	200	1200	NS	NS	NS
58	2	240	280	560	160	360	1360	600	280	NS	720	800	480	1000
59	2	240	120	40	200	680	360	240	120	40	120	40	80	600
63	2	160	40	40	560	280	640	1360	600	360	680	720	1400	2440
69	2	120	400	160	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
71	2	40	160	640	4360	2320	6560	6880	**3240**	**0**	80	2040	4040	5880
74	2	80	40	160	240	240	520	560	1120	200	400	1360	1720	2200
75	2	200	280	480	600	440	840	160	240	320	360	840	520	1880
76	2	0	360	680	1280	320	2120	440	760	880	800	2000	1280	1640
80	2	160	160	360	NS	360	1040	560	480	680	640	560	800	720
96	2	0	NS	400	200	440	760	440	600	0	880	NS	NS	NS

Table 8 - Tabulated Blood Haemoglobin Concentrations (g/dl).
NS denotes No Sample, number in **BOLD** denotes sheep drenched for low blood haemoglobin content

Animal ID	Group	Day 0	Day 21	Day 42	Day 56	Day 70	Day 84	Day 98	Day 112	Day 126	Day 140	Day 154	Day 168
1	1	9.6	9.1	9.3	8.6	7.9	8.7	8.6	9.9	8.1	8.2	9.3	10.2
3	1	9.1	8.2	8.3	7.5	7.0	7.2	8.1	8.6	8.7	9.3	9	9.2
6	1	9.5	10.4	9.5	9.5	NS	10.0	10.3	9.5	10.7	10.9	9.5	10.2
15	1	11.0	10.7	9.8	10.7	9.8	10.8	10.8	11.3	10.4	10.3	11.2	9.8
17	1	8.6	8.7	7.4	**5.6**	8.8	8.9	8.8	7.1	**5.6**	8.6	9.7	10.2
18	1	10.2	9.6	9.5	9.2	8.2	8.1	8.1	8.9	7.3	7.8	8	9.4
20	1	11.3	11.3	10.2	10.3	9.1	11.5	10.1	10.4	9.9	NS	NS	NS
29	1	9.7	9.6	9.6	9.3	8.3	9.1	9.2	10.1	9.1	NS	NS	NS
32	1	9.3	9.1	8.2	7.5	**6.4**	9.8	9.9	8.7	6.6	**6.2**	10	11.6
34	1	9.5	8.8	8.1	8.2	8.2	8.6	9.1	8.9	8.7	9.1	8.5	10.1
35	1	9.0	9.8	8.9	8.1	8.5	7.9	6.9	10.3	10.7	10.5	10.5	10.8
36	1	9.4	9.4	9.2	9.1	8.0	8.9	9.3	9.7	10.3	10.3	9.2	10.7
37	1	10.2	9.8	7.9	9.0	8.1	8.5	9.4	9.8	10.5	9.6	8.8	9.5
42	1	10.3	10.1	10.2	9.1	8.5	7.3	6.4	10.5	9.6	9	8.4	8
43	1	8.6	8.6	8.3	8.7	NS	NS	8.0	8.1	**4.0**	**6.3**	8	9.6
44	1	10.3	9.9	9.2	8.8	8.6	9.6	10.5	9.4	8.6	9	9.6	9
48	1	9.9	10.0	NS	9.4	8.3	8.9	9.6	9.3	9.0	9	8	8.5
51	1	10.7	10.1	9.6	8.1	7.6	8.9	10.0	9.2	9.0	10.1	9.3	10.5
52	1	9.5	9.3	9.3	9.5	7.9	9.0	8.3	8.5	9.4	7.8	8.7	8.7
54	1	11.9	10.0	10.9	10.2	9.3	9.5	10.6	10.2	10.1	11.6	11	11.3
55	1	6.9	**6.2**	8.2	7.1	6.7	7.3	8.8	7.8	7.1	7.7	7.9	7.2
57	1	10.0	10.7	9.8	9.2	8.5	9.8	9.2	9.1	7.6	NS	7.5	8.2
60	1	10.5	9.9	10.0	9.5	8.6	9.0	9.4	9.0	7.7	**6.5**	10	10.9
61	1	11.7	10.8	9.9	9.1	8.2	9.8	9.8	8.6	8.8	NS	NS	NS
62	1	10.8	9.8	9.7	9.8	8.8	8.9	10.1	9.4	9.3	9.9	8.2	8.6

66	1	10.2	10.2	11.0	9.7	8.6	10.8	9.5	8.3	8.0	8.1	8.8	9.5
67	1	10.4	10.6	10.4	10.2	9.5	10.3	9.7	10.3	7.7	10.5	10.7	10.5
70	1	9.5	9.2	8.2	8.0	6.9	7.8	7.4	9.2	9.3	10.4	9	9.3
77	1	10.8	11.0	9.0	10.1	9.4	9.6	11.0	11.9	8.4	NS	NS	NS
78	1	10.5	11.0	10.7	10.1	7.5	8.4	9.4	10.2	11.2	11.5	10.5	10.5
2	2	9.2	8.1	8.2	7.4	9.2	8.9	9.2	9.3	8.5	9.5	9.3	10.4
4	2	9.8	9.8	9.6	9.9	9.3	9.0	8.4	9.5	8.8	9	8.5	10.1
7	2	10.9	7.4	10.6	9.8	9.9	8.7	8.2	**5.3**	9.7	11.7	11.4	10.2
8	2	9.6	8.7	8.0	8.7	8.9	9.1	8.9	9.0	8.2	8.8	8	9.1
10	2	9.7	10.3	10.0	10.9	9.4	10.4	10.2	11.1	10.2	9.6	9.3	10.7
12	2	8.4	8.7	9.0	8.7	8.9	8.4	9.5	9.5	8.9	9.1	8.7	8.4
13	2	8.1	8.9	7.1	**6.3**	NS	9.8	10.9	10.0	8.5	NS	6.5	6.1
14	2	10.0	10.3	10.4	10.7	9.6	10.8	10.8	11.4	9.5	10.8	10.7	11.2
22	2	10.1	10.3	7.2	9.0	9.5	9.0	9.4	9.6	9.0	8.3	9	9.9
23	2	8.9	8.2	7.9	8.5	7.5	NS	NS	NS	NS	NS	NS	NS
24	2	**6.1**	8.6	9.8	9.1	8.9	8.6	9.0	9.2	9.3	NS	NS	NS
26	2	9.7	9.3	9.6	8.8	8.8	9.0	9.0	9.7	9.3	8.9	8.9	8.9
27	2	10.3	10.1	9.9	9.8	9.1	8.5	9.3	9.4	9.3	8.6	8.5	8.9
30	2	9.5	9.1	9.7	10.6	9.2	9.3	10.2	10.1	8.7	9.8	10.7	10.2
33	2	10.4	10.3	10.5	9.5	9.5	10.2	9.8	9.2	9.1	8.6	9	9.5
38	2	11.2	10.7	11.0	11.3	10.3	11.3	10.7	9.4	10.6	10.8	10.4	11.1
41	2	8.1	9.6	9.2	9.0	8.5	8.2	8.5	9.1	8.3	10	8.4	9.1
47	2	9.4	9.2	9.5	8.8	8.4	8.9	8.6	9.1	9.5	9.4	9.1	9.9
49	2	9.8	8.9	10.1	10.0	10.2	9.6	10.5	10.9	10.3	9.5	8.6	8.7
53	2	6.9	9.8	10.1	9.6	9.1	8.8	9.2	9.5	8.5	NS	NS	NS
58	2	9.9	9.2	9.5	10.0	8.9	9.3	9.4	9.6	9.7	10	9.5	10.1
59	2	10.6	10.4	10.7	10.7	10.5	10.2	11.1	10.3	10.3	11.3	10.3	11
63	2	9.7	10.7	10.3	10.7	8.8	8.6	9.3	11.5	10.0	10.7	9.6	10.3
69	2	8.4	7.7	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

71	2	10.1	9.5	8.8	9.8	8.6	8.3	8.4	10.4	10.0	9.7	9.2	8.6
74	2	7.5	10.9	10.6	11.1	9.3	9.6	10.4	10.1	9.7	8.8	8.8	9.5
75	2	8.9	8.5	8.0	9.2	8.6	7.9	9.3	9.5	9.1	8.1	8.7	9.1
76	2	10.4	10.5	9.7	10.0	9.6	10.1	11.1	11.4	10.6	10.5	10	10.9
80	2	11.5	11.4	10.4	10.7	10.1	10.7	11.2	10.9	10.5	10.7	9.9	12.4
96	2	10.5	9.8	8.4	9.2	9.2	10.0	8.9	9.8	9.2	NS	NS	NS

Table 9- Tabulated ELISA titres

Group	Sheep#	Day 0	Day 14	Day21	Day 42	Day 56	Day 70	Day 84	Day 98	Day 112	Day 126	Day 140	Day 154	Day 168
Vaccinates	2	2,795	6,547	5,827	4,460	8,257	7,716	4,449	19,772	8,291	8,657	26,648	15,742	8,611
	4	2,951	7,953	6,189	4,584	13,036	8,644	6,322	62,861	26,650	13,385	48,346	30,971	22,411
	7	444	6,149	4,625	2,891	9,884	4,708	3,139	16,968	7,950	5,346	29,654	11,134	7,417
	8	3,520	3,108	3,120	3,383	8,449	5,007	4,177	9,302	5,643	4,942	16,819	10,081	7,063
	10	175	4,438	2,598	1,669	4,081	2,644	1,599	4,999	4,120	2,936	9,687	5,425	4,620
	12	3,939	10,362	6,753	5,638	8,562	7,846	5,971	17,234	10,610	6,956	14,993	9,327	7,431
	13	2,634	3,991	3,539	1,074	5,164	NS	2,839	16,871	7,009	4,876	NS	4,903	4,334
	14	1,217	8,083	5,462	3,973	7,093	6,450	1,737	11,572	6,058	4,697	10,128	6,097	5,053
	22	1,458	4,937	4,103	3,686	9,257	5,824	5,120	19,073	5,953	5,462	18,279	10,547	7,419
	23	1,596	2,939	1,637	2,677	17,066	6,796	NS	NS	NS	9,241	NS	NS	NS
	24	3,355	30,467	17,021	7,133	16,077	12,270	6,109	83,192	10,396	NS	NS	NS	NS
	26	1,283	5,578	4,287	3,341	6,210	4,485	3,907	8,875	6,720	5,279	4,591	4,718	4,593
	27	4,457	5,981	4,973	5,412	84,917	51,089	30,451	59,321	32,203	30,530	19,169	18,873	14,811
	30	3,554	65,937	6,846	5,679	8,424	6,824	4,914	21,810	6,673	6,932	8,161	14,068	11,865
	33	2,188	3,881	3,435	3,492	10,961	7,906	5,810	15,361	7,124	8,780	43,398	21,524	15,741
	38	1,379	1,626	1,282	1,602	5,489	3,261	2,168	7,077	6,325	4,086	9,217	6,775	5,602
	41	1,972	1,614	1,537	2,769	6,321	4,071	3,635	5,710	3,837	3,913	9,648	8,177	4,976
	47	1,203	5,821	4,620	3,668	10,632	6,181	3,697	10,426	6,050	5,301	18,456	9,769	7,648
	49	1,313	8,892	5,532	4,141	5,366	3,822	3,013	7,378	5,436	5,117	4,481	4,842	3,651
	53	813	2,044	1,610	1,170	4,663	2,645	1,939	13,115	7,491	4,465	NS	NS	NS
	58	1,873	3,031	2,004	3,242	16,454	7,819	3,967	15,412	6,024	4,927	4,159	8,497	6,692
	59	185	3,340	2,055	1,360	6,291	5,001	2,986	13,014	5,461	4,412	44,827	22,579	11,547
	63	6,370	32,672	23,704	15,877	39,916	30,581	10,170	55,868	33,991	27,466	11,959	23,750	10,590
	69	2,382	10,784	9,288	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	71	1,441	3,773	2,929	2,204	6,258	4,281	3,470	10,887	5,717	4,302	9,741	10,248	5,472
	74	1,919	12,572	10,026	4,961	11,198	6,139	5,514	14,384	8,963	5,403	16,455	7,860	7,789

	75	4,051	14,274	11,256	8,659	12,802	8,525	8,507	23,129	15,733	9,487	36,463	26,030	14,409
	76	948	8,337	5,187	3,333	7,999	5,064	3,755	12,284	6,676	4,131	12,160	7,130	5,183
	80	2,780	3,548	4,095	4,076	5,593	3,971	3,814	6,604	5,143	4,589	8,843	6,377	7,693
	96	192	4,057	1,814	1,093	4,489	3,375	2,591	11,280	5,880	5,273	NS	NS	NS
mean		**2,146**	**9,558**	**5,578**	**4,043**	**12,445**	**8,319**	**5,206**	**20,492**	**9,576**	**7,532**	**18,178**	**12,218**	**8,505**
sem		263	2,352	880	538	2,881	1,859	1,005	3,713	1,503	1,222	2,693	1,496	895
Group	**Sheep#**	**Day 0**	**Day 14**	**Day21**	**Day 42**	**Day 56**	**Day 70**	**Day 84**	**Day 98**	**Day 112**	**Day 126**	**Day 140**	**Day 154**	**Day 168**
controls	1	0.31						2						196
	3	304.91						724						2,003
	6	0						2						337
	15	26.87						255						3,005
	17	0						14						488
	18	131.91						1,171						1,702
	20	1.43						396						NS
	29	0.51						591						NS
	32	0						502						1,999
	34	38.35						1,172						2,043
	35	0.11						0						48
	36	99.73						943						1,249
	37	0.08						683						991
	42	140.66						411						1,933
	43	19.37						235						1,873
	44	206.55						893						5,981
	48	0.02						20						85
	51	0.03						240						1,000
	52	4.77						345						2,534
	54	0						413						1,777
	55	10.2						590						2,322

	57	31.81						807						1,341
	60	584.14						1,027						3,329
	61	0.65						554						NS
	62	0.1						528						1,245
	66	5.75						1,045						1,607
	67	0						0						0
	70	200.62						487						2,034
	77	0.54						784						NS
	78	23.43						205						387
	mean	**61**						**501**						**1,596**
	sem	23						67						251

Table 10 - Tabulated Larval Differentiation

Larval Differentiation								
Group	**Day**	**Treatment**	**Haem**	**Trich**	**Ost**	**Coop**	**Oes**	**Total**
1	0	Neg. controls	61	22	17	0	0	100
2		Vaccine	38	19	43	0	0	100
1	14	Neg. controls	63	19	18	0	0	100
2		Vaccine	66	15	19	0	0	100
1	21	Neg. controls	84	8	8	0	0	100
2		Vaccine	49	4	46	1	0	100
1	42	Neg. controls	97	3	0	0	0	100
2		Vaccine	79	13	7	0	1	100
1	56	Neg. controls	87	7	6	0	0	100
2		Vaccine	69	11	20	0	0	100
1	70	Neg. controls	91	6	3	0	0	100
2		Vaccine	72	10	14	2	2	100
1	84	Neg. controls	46	48	3	0	3	100
2		Vaccine	49	45	0	0	6	100
1	98	Neg. controls	89	11	0	0	0	100
2		Vaccine	36	44	14	1	5	100
1	112	Neg. controls	94	5	1	0	0	100
2		Vaccine	24	55	12	0	9	100
1	126	Neg. controls	87	12	0	0	1	100
2		Vaccine	51	47	2	0	0	100
1	140	Neg. controls	92	3	5	0	0	100
2		Vaccine	57	22	16	1	4	100
1	154	Neg. controls	96	3	1	0	0	100
2		Vaccine	69	26	5	0	0	100
1	168	Neg. controls	71	29	0	0	0	100
2		Vaccine	32	63	5	0	0	100

APPENDIX 4

STATISTICAL ANALYSIS

1. *Haemonchus* egg counts

Log transformed *Haemonchus* egg counts from control and vaccinated lambs were compared on each sampling from Day 42 using two tailed t tests and Welch's correction for unequal variances. This was done using the software package "Graphpad", the output from which follows:-

Table Analyzed	**Kingstown log Hc epgs d42**
Column A	Control D 42
vs	vs
Column B	Vaccine D 42

2. Bodyweights

The bodyweights of control and vaccinated lambs were compared on each weighing day using two tailed t tests and Welch's correction for unequal variances. This was done using the software package "Graphpad", the output from which follows:-

Table Analyzed	**Kingstown for t test d0**
Column A	C0
vs	vs
Column D	V0

3. Blood Haemoglobin concentrations

The Haemoglobin concentrations of control and vaccinated yearlings were compared on selected sampling days using two tailed t tests and Welch's correction for unequal variances. This was done using the software package "Graphpad", the output from which follows:-

Table analyzed	**kingstown hb d0**
Column a	cont d 0
Vs	vs
Column n	v day 0

APPENDIX 5

WEATHER DATA

Mean Monthly Rainfall (mm) measured at Kingstown post office weather station

	Jan	Feb	Mar	Apr	Oct	Nov	Dec
2012					19.8	*68.8*	*78.0*
2013	*158.4*	*64.0*	*71.6*	*3.2*			

APPENDIX 6

ANIMAL ETHICS AUTHORITY

APPENDIX 7

TRIALPAK

Section	Title	Page
1	Protocol	
2	Amendments, Deviations and Notes to File	
3	Drug Reconciliation	
4	Trial Log	
5	Signature Log	
6	Animal Information Record	
7	Owners Consent and Livestock Compensation	
8	Scale Verification and Bodyweight Records	
9	Allocation	
10	Treatment Data	
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16	Sample Dispatch and Receival Records	
17	Animal Accountability	