final report

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Development of a commercial vaccine for *Haemonchus contortus*, the Barber's Pole Worm

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Abstract

Barbervax, a vaccine for Barber's Pole worm, was registered for use in Australian lambs in October 2014. The main object of the present project was to evaluate the efficacy and safety of the vaccine for ewes, with a view to extending registration across the whole flock. Three field trials conducted in the Northern Tablelands of NSW showed that Barbervax could curb *Haemonchus* egg counts in ewes both before and after weaning, especially if they had been vaccinated in a previous season. Reducing egg output during lactation is important because this is the main source of infection for the next generation of sheep, while lower egg counts post weaning will reduce anaemia and deaths in ewes during late summer and autumn, the peak season for Barber's Pole worm disease. A large scale safety trial with some 600 ewes was favourable in that it confirmed that any adverse effects of using Barbervax were mild and transient. A secondary objective was to determine whether Barbervax could be useful for producers in the Northern Slopes of NSW. Graziers in this region would be reluctant to adopt the 5 vaccination schedule recommended for Barbervax in the Northern Tablelands because the effort and expense of mustering on their more extensively grazed properties would be prohibitive. The trial was encouraging, because a strong antibody response was detected post weaning, but unfortunately inconclusive, because drought prevented any significant challenge. This information together with earlier results from trials with yearling sheep has been compiled and submitted to the APVMA in order that authority for using Barbervax across entire flocks can be obtained.

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. Barbervax, a vaccine for *H.contortus*, has recently been approved for sale in Australian lambs, the main objective of the present work was to determine whether it was also effective in ewes.

The present project was the third 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 has been developed at the Moredun Research Institute in Scotland following some 20 years work and is manufactured by one of its subsidiaries, Wormvax Australia, at the Department of Food and Agriculture, Western Australia laboratory in Albany WA in collaboration with local parasitologists.

This first part (Project B.AHE.0068) of the overall objective was concerned with obtaining a Good Manufacturing Practice (GMP) licence for the vaccine and with determining vaccine efficacy and safety for lambs in field trials. Most of the trials were done in New England, NSW where *Haemonchus* is endemic and a serious problem (two trials not funded by MLA were also done in WA). The results were successful and culminated in the registration of Barbervax for use in lambs by the Australian Pesticide and Veterinary Medicines Authority (APVMA) on 1st October 2014.

The second part (Project B.AHE.0214) of the overall objective showed that the vaccine afforded lambs an epidemiological benefit by reducing pasture contamination with infective worm larvae. In addition, it showed that the vaccine was effective in yearling sheep

The main aim of the present project (B.AHE.0232), the third part of the overall objective, was 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 ewe deaths due to Barber's Pole worm are not uncommon.

The results have been favourable and a second dossier containing the data from B.AHE.0232 and B.AHE.0214 was submitted to the APVMA in December 2014. Therefore, if approval of the second dossier is obtained, farmers will be able to use the vaccine in lambs, yearlings and ewes. By this means it is hoped that Australian farmers will have a new tool to combat Barber's Pole worm across their entire flock.

Contents

1. Background

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 being one possibility. 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. Objectives

This report describes the third of three projects aimed at making an effective Barber's Pole worm vaccine commercially available for Australian sheep producers. The first (B.AHE.0068) and second (B.AHE.0214) projects have been reported separately already.

This project was mainly concerned with determining whether the vaccine could 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 ewe deaths due to Barber's Pole worm are not uncommon. A large scale safety trial with ewes was also conducted.

A secondary objective was to determine whether Barbervax could be useful for producers in the Northern Slopes of NSW. Graziers in this region would be reluctant to adopt the 5 vaccination schedule recommended for Barbervax in the Northern Tablelands because the effort and expense of mustering on their more extensively grazed properties would be prohibitive.

Last but by no means least, the third objective was to compile the findings from this and the previous (B.AHE.0214) into a dossier for submission to the APVMA. This was achieved in December 2014.

3. Methodology

The methods used to conduct the various sheep trials are detailed in their specific reports as described in Appendices 6.1, 6.2, 6.3, 6.4 and 6.5. These reports are truncated versions of the those submitted to the APVMA.

4. Results

4.1 Vaccine efficacy trials with ewes

Pregnant ewes lose their naturally acquired immunity to worms around lambing time, shedding eggs which become the main source of pasture contamination for their offspring. Therefore it was of particular interest to know whether Barbervax could curb *Haemonchus* egg output from the ewes before weaning and, if so, what effect would it be predicted to have on the build-up of infection in their lambs

Three trials were conducted, each on a different property. On two of these a group of pregnant ewes was available which had received two courses of Barbervax previously, the first when they were lambs, the second when they were hoggets. The effect of giving Barbervax to these Previously Vaccinated animals was compared with that of age-matched pregnant ewes vaccinated for the first time, the so-called First Vaccinated group. The detailed methods and results of these trials are presented in Appendices 6.1, 6.2 and 6.3.

In an attempt to ensure high levels of circulating vaccine antibodies during the periparturient period, the First Vaccinated group was injected twice with Barbervax approximately one month apart before lambing. Because the serology from preceding yearling trials had predicted that the Previously Vaccinated group would mount a secondary response, these ewes were given the second of these injections only. Further boosting of all the vaccinates occurred at lamb marking time, at weaning and at six week intervals thereafter till the end of the season. For simplicity and to ensure equal exposure to natural infection vaccinated and control ewes grazed the same paddock. Worm control in the lambs was attained by repeated drenching.

Barbervax did reduce ewe egg output both during lactation and the trial as a whole, but the effect was superior in the sheep which had received previous courses of the vaccine (Table 2). On the two properties where significant precautionary drenching was required for the control sheep, significantly less was needed for the vaccinates, especially those which had received Barbervax when younger. In other words Barbervax had an impact both on the potential build-up of *Haemonchus* on the paddock but also on the health of the ewes themselves.

Table 2. Percent reduction in *Haemonchus* egg counts of vaccinated ewes

		Period	
Trial Site	**Group***	**Lactation only**	**Whole trial**
Kingstown	FV	50	61
Dundee	FV	18	32
	PV	55	55
CSIRO	FV	29	21
	PV	71	73

*FV = First Vaccinated

*PV= Previously Vaccinated

The downstream benefit of using Barbervax in both ewes and their lambs was predicted by computer modeling done by Dr Robert Dobson in a parallel project funded by the European Union. When the scenario of 50-70% reduction in egg output of the previously vaccinated sheep ewes (Table 2 above) combined with an 80% reduction in lamb egg output (the average obtained in the previously reported field trials) was simulated, the overall benefit was substantial (see Tables 3 and 4 below).

Table 3 shows the benefit of vaccination by comparison with unvaccinated lambs and ewes that received a persistent anthelmintic treatment regimen.

Vaccination Protection		Animal	mean Hc	haemonchosis
Ewe	Lamb	%Deaths	epg	years/20
50-80%	70-80%	1.8	142	1.6
Un-vacc	Un-vacc	22.0	561	12.5
vaccine benefit		**20.2**	**419**	**10.9**

Table 4 shows the benefit of vaccination by comparison with unvaccinated lambs and ewes that received a short acting anthelmintic treatment regimen.

Vaccination Protection		Animal	mean Hc	haemonchosis
Ewe	Lamb	%Deaths	epg	years/20
50-80%	70-80%	1.8	142	1.6
Un-vacc	Un-vacc	29.3	924	13.5
vaccine benefit		**27.5**	**783**	**11.9**

It was concluded that a course of Barbervax could aid in the control of *Haemonchus* in adult ewes especially if they had received a course of vaccine in an earlier season. More importantly perhaps, the combined effect gained by vaccinating both ewes and their lambs was likely to provide substantial benefit to the flock as a whole.

4.2 Large scale safety trial with ewes

The results detailed in Appendix 6. 4 showed that apart from a mild rise in rectal temperature one to 3 days after administration Barbevax provoked no detectable adverse reactions.

4.3 Efficacy trial with extensively raised lambs

Here a vaccination regime consisting of injections at marking and at weaning 10 weeks later, stimulated a strong post weaning secondary antibody response which was on a par with protective levels observed in New England lambs during 2011-12. Unfortunately a natural challenge infection failed to develop because of the drought conditions and so it is uncertain how protective the observed response would have been.

5. Discussion/conclusions

The efficacy trial with extensively raised lambs near Narrabri was encouraging from a serological perspective, but inconclusive thanks to the lack of worm challenge. Fortunately however, there was sufficient rain in the New England district to provide an adequate challenge to test Babervax efficacy in the three trials with ewes. These trials showed that a course of Barbervax significantly reduced ewe egg counts both before and after weaning, but the effect was stronger and more reliable if the animals had been immunised in a previous season. The efficacy observed would probably have been increased if the vaccinates had grazed separately from the unvaccinated control ewes, as users of the vaccine would be strongly advised to do.

Computer simulations indicated that when the reduction in egg count observed in ewes which had been vaccinated in an earlier season was combined with that previously observed in lambs, the epidemiological benefit to the flock was substantial.

Meanwhile a large scale safety study indicated negligible adverse reaction to the vaccine.

It is suggested from the results reported from the three MLA Barbervax projects (B.AHE.0068, 0214 and 0232) completed to date that adopters should phase the vaccine in to their flocks over consecutive years as follows:-

Year 1 – vaccinate lambs only;

Year 2 – vaccinate lambs and replacement hoggets which had been immunised in Year 1 when lambs;

Year 3, vaccinate lambs, replacement hoggets (immunised in Year 2 when lambs) and maiden ewes ((immunised in Year 1 as lambs and Year 2 as hoggets). This progression would continue so that after a few years all ewes in the flock would be immunised.

As for wool growing wethers and rams, following their first course of Barbervax as lambs, four injections given annually 6 weeks apart starting just before the high risk *Haemonchus* period (similar to that given to the yearlings in Project B.AHE.0214) should provide adequate cover.

6. Appendices

Appendix 6.1. CSIRO ewe efficacy trial report

CSIRO Livestock Industries

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

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Field test of Barbervax in lambing and lactating Merino ewes.

Background to earlier Barbervax trials conducted at CSIRO Chiswick/Arding

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 Barbervax 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

During the 2012-2013 summer, a second trial with weaner lambs was conducted, but this time vaccinates and controls grazed apart on adjacent replicated paddocks. The protective effect of the vaccine was greater under these circumstances.

A third trial was run over the 2012-2013 summer using some of the 2011-2012 trial sheep which were now yearlings. Thirty of these animals (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 observed after the vaccinates received their first immunisation 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 yearlings (which had previously been vaccinated as lambs) against Haemonchosis.

The present trial of Barbervax in breeding ewes.

The performance of three groups of age matched breeding ewes grazing the same pasture was compared. Two groups were vaccinated whereas the third were unvaccinated control animals. One vaccinated group had received earlier courses of Barbervax as lambs and yearlings, the other was vaccinated for the first time. Those vaccinated for the first time were injected some 6-8 weeks (39 to 53 days) before lambing, 4 weeks later (11-25 days pre-lambing), at marking (when 26-40 days old) and at weaning, followed by two more boosts 6 weeks apart. Those previously vaccinated were given the same Barbervax regime, except that the first injection was omitted.

It was very clear that the Previously Vaccinated ewes were significantly protected against *Haemonchus* compared to the Control animals, measured in terms of reduced egg output and anaemia both before and after weaning. Their degree of protection was superior to that observed in the ewes vaccinated for the first time.

Field test of MRI Haemonchus vaccine efficacy in Merino ewes.

1. **Objective**
   To assess the efficacy of "Barbervax", the Moredun Research Institute *Haemonchus* vaccine, for Merino ewes over the high risk period of exposure to this parasite including during the periparturient and lactating periods. If favourable, the results will extend the scope of the registration package for this commercial product to breeding ewes.

2. **Justification**
   Haemonchus contortus is the major nematode pathogen in high summer 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 grazing lambs and yearlings during the summers of 2011-2013, but its protective ability for breeding ewes remained untested.

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	***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

5. Study Dates

Start date (Animal Phase):	4 September 2013
Finish date (Animal phase):	9 April 2014
Finish date (laboratory phase):	12 May 2014

6. Study Design

The trial contained 66 age matched two year old Merino ewes from the same Elite CSIRO line. All had been synchronised and were due to lamb over a two week period in October 2013. They were allocated to 3 treatment groups named Previously Vaccinated, First Vaccinated and Control.

The Previously Vaccinated group consisting of 16 ewes which had been vaccinated with Barbervax during the 2011-2012 and 2012-2013 summers when they were lambs and yearlings, respectively.

The First Vaccinated and Control groups contained 25 sheep each. The Control group was not vaccinated with Barbervax. The First Vaccinated group received their first dose of Barbervax approximately 5 to 7 weeks before lambing and a second injection four weeks later when the Previously Vaccinated group were also vaccinated. Thereafter both vaccinated groups were boosted together three more times at 6 to 7 week intervals.

All trial animals were run together as a single mob with their lambs. The lambs were marked when they were 3 to 6 weeks old and weaned when aged approximately 14 weeks, so that the ewes remained on the paddock till the end of the trial. The lambs were drenched 5 times between marking and weaning to ensure that only ewes contributed to the larval challenge on the paddock.

A group of 6 yearling tracer sheep also co-grazed the paddock. These animals were given a short acting anthelmintic at approximately 6 week intervals during the trial. Their purpose was to monitor pasture infectivity, evident if their faecal egg counts became positive between the drenches they received.

Individual faecal samples were collected from all ewes and the tracers at 2 week intervals throughout the trial 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. Ewe liveweights were recorded on Day 28 of the trial and at marking, weaning and trial end. Lambs were weighed at birth, marking and weaning. 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 ewe was <7.5 g/100mL (equivalent PCV 22%) or the FEC was >10,000, that sheep was treated immediately with a short-acting anthelmintic effective against *Haemonchus* at the manufacturer's recommended dose rate. The animal remained with the trial flock after such treatment.

7. INVESTIGATIONAL & CONTROL PRODUCTS

a. Investigational Veterinary Product (IVP):

Name:	BarberVax	Batch No.:	HCD220311C-009
Composition:	*Haemonchus* antigen and saponin adjuvant	Expiry Date:	April 2015
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^{o}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 for the IVP is attached.

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

8. Treatment

a. **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).
b. **Treatment frequency**: On five occasions on Days 0, 28, 77, 133 and 161.
c. **Dose**: 1 mL per ewe.

9. Schedule of events

Date	Activities	Days after V1
15/05/2013	Sponges out, rams IN	-112
27/05/2013	Rams OUT	-100
22/07/2013	All ewes vaccinated against Clostridia (6-in-1)	-44
31/07/2013	Scan for pregnancy	-35
19/08/2013	Tracers drenched 3 mLs Zolvix	-16
21/08/2013	Moved Tracers to trial area	-14
04/09/2013	Vaccinated 25 Group 2 ewes, blood & faecal samples taken	0
02/10/2013	Vaccinated Group 1 and Group 2 ewes, blood & faecal samples taken, Drenched Tracers. Ewes given side brands for identification and split into 3 groups for lambing. Divided tracers into 3 groups as well (4 with group 1, 3 each in other two groups). All animals moved to H lane. Commenced hand feeding.	28
10/10/2013	First lamb born - see detailed records in Lambing sheet.	36
27/10/2013	Finished lambing	53
28/10/2013	Ewes and lambs combined into one grazing group (plot 6 + laneway).	54
31/10/2013	Ewes and lambs moved to plots 2 and 3	57
06/11/2013	Blood & faecal samples taken from ewes, faeces from tracers	63
20/11/2013	Marked lambs, blood & faecal samples taken from ewes, faecal samples from Tracers. Vaccinated Groups 1 and 2 Ewes, Drenched Tracers Triguard	77
21/11/2014	Weighed ewes and lambs, lambs drenched with Rycazole and Cydectin	78

Date	Activities	Days after V1
04/12/2013	blood & faecal samples taken from ewes, faecal samples from Tracers, drenched lambs Rycazole and Triguard	91
18/12/2013	blood & faecal samples taken from ewes, faecal samples from Tracers, drenched lambs with Zolvix	105
03/01/2014	Blood & faecal samples taken from ewes, faecal samples from Tracers,Vaccinated Group 1 and 2 Ewes, Drenched tracers (Zolvix) & lambs (Rycazole and Cydectin)	121
15/01/2014	Blood & faecal samples taken from ewes, faecal samples from Tracers,Vaccinated Group 1 and 2 Ewes, Drenched lambs (Rycozole)	133
16/01/2014	Drenched 3 ewes (Triguard)	134
29/01/2014	Blood & faecal samples taken from ewes, faecal samples from Tracers, Weaned and Drenched lambs (Rycozole & Zolvix), weighed ewes and lambs. Drenched 7 ewes. Lambs removed from trial.	147
12/02/2014	Blood & faecal samples taken from ewes, faecal samples from Tracers, Vaccinated Group 1 and 2 Ewes, Tracers drenched (Rycozole & Triguard). 5 ewes drenched (Triguard)	161
26/02/2014	Blood & faecal samples taken from ewes, faecal samples from Tracers.	175
12/03/2014	Blood & faecal samples taken from ewes, faecal samples from Tracers.	189
26/03/2014	Blood & faecal samples taken from ewes, faecal samples from Tracers. 2 ewes drenched (Triguard)	203
09/04/2014	Blood & faecal samples taken from ewes, faecal samples from Tracers. All ewes and tracers drenched (Triguard, Rametin and Flukazole). Ewes weighed.	217

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^{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.

12. Assessment of Effects

a. **Sheep liveweights**: Ewe weights were recorded on Day 28 of the trial and at marking, weaning and trial end. Lambs were weighed at birth, marking and weaning 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.
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.

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

13. Statistical Analyses

Faecal egg counts, blood haemoglobin concentrations and bodyweights were compared between groups by analysis of variance, whereas the number of precautionary drenches was compared by Fisher's exact test. The faecal egg counts were cube root transformed prior to analysis.

14. Results

a. Deaths

One ewe from the first vaccinated group (#7430) died before day 63 of the trial due to accidental suffocation in an over-packed race. Its data was excluded from the analysis.

b. Lambing success and number of ewes used in the study

There were 66 ewes at the start of the trial but 8 did not raise a lamb (2 in the Previously Vaccinated group and 3 in each of the First Vaccinated and Control groups), either because they were not pregnant, or because their lamb was born dead or died soon after birth. These 8 sheep remained with the rest of the flock throughout the trial but their data was excluded from the analysis.

Of the 57 surviving ewes which raised a lamb (all singles), 14 had been previously vaccinated, 22 were vaccinated for the first time and 21 were in the Control group.

c. Types of comparison made

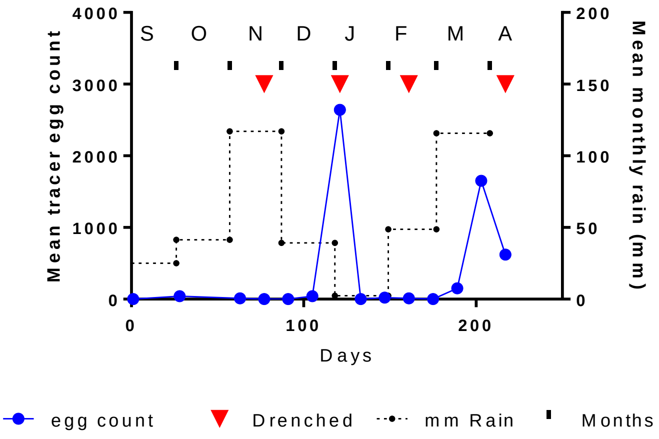
It was of particular interest to know how well the vaccine performed during the "periparturient" phase of the trial because worm eggs shed during lactation are an important source of infection for the next generation of lambs and hence the general epidemiology of Haemonchosis in a flock. Therefore the degree of protection attained from lambing to weaning is presented in addition to that calculated for the whole duration of the trial.

d. Rainfall and pasture infectivity

The 2013-2014 summer was exceptionally dry in New England. Table A6 compares the monthly rainfall recorded at Armidale Airport (a few Km from the trial site) during the trial with the historical monthly average.

The mean egg counts of the tracer lambs are plotted in Fig 1 relative to rainfall. If infective larvae had been available continuously, positive egg counts would be expected at or just before each drench, but this was not the case at the November or February drench points, indicating that insufficient rain for egg to worm larval development had fallen since the previous dose of anthelmintic.

Fig 1. Rainfall and pasture infectivity

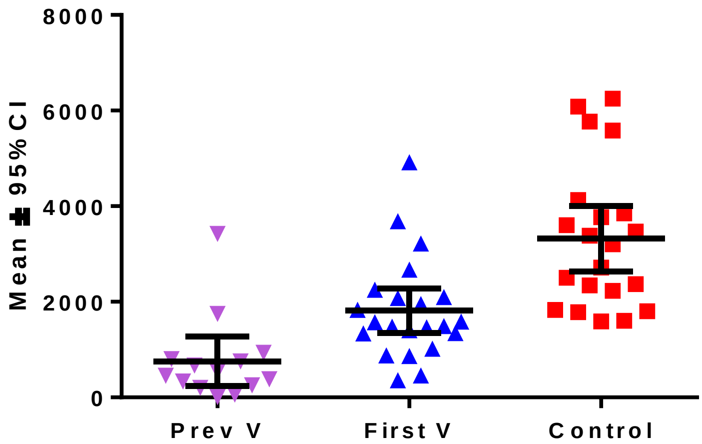


15. *Haemonchus* Egg Counts

i) From lambing to weaning

During lactation *Haemonchus* egg counts were significantly reduced in both groups of vaccinated sheep compared to controls (Fig 2) although the protective effect was significantly better in the Previously Vaccinated group.

Fig 2. Individual ewe counts averaged over lactation



ii) Over the whole trial

Haemonchus egg counts were significantly reduced in the Previously Vaccinated sheep compared to those vaccinated for the first time and the controls (Fig 3). However, over the duration of the trial, no significant difference was detected between the counts of the First Vaccinated group and the Controls.

A single "non-responder" (defined as a vaccinated animal with a mean egg count greater than the 95% lower confidence limit of the control group) was identified in the Previously Vaccinated group.

Fig 3. Individual ewe counts averaged over the trial

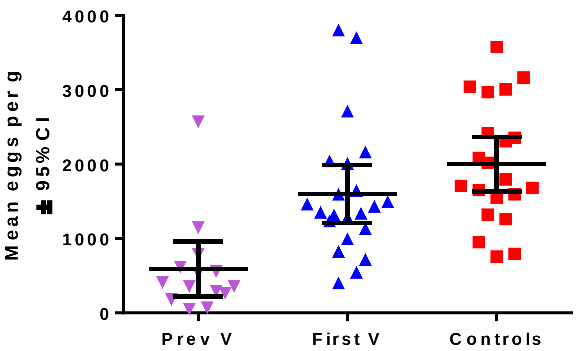


Table 1		**PV**	%Prot-	**FV**	%Prot	**control**		**P value after ANOVA**	
Event	**Day**	**Mean**	ection	**Mean**	ection	**Mean**	**PV vs FV**	**PV vs Cont**	**FV vs Cont**
V1	**1**	41		71		**24**	n/a	n/a	n/a
V2	**28**	35		170		**56**	n/a	n/a	n/a
	63	411	59.0	1010	-0.6	**1004**	***	***	ns
V3	**77**	547	66.8	1671	-1.4	**1648**	***	***	ns
	91	381	74.1	800	45.6	**1471**	ns	***	*
	105	187	85.8	603	54.1	**1314**	**	****	*
V4	**121**	1449	77.3	4729	26.0	**6390**	****	****	ns
	133	1112	82.9	2502	61.5	**6499**	*	****	*
weaning	**147**	1945	51.7	3182	21.0	**4027**	ns	ns	ns
V5	**161**	274	85.7	1259	34.2	**1915**	ns	ns	ns
	175	280	61.3	775	-7.0	**724**	ns	ns	ns
	189	71	84.5	298	34.4	**455**	*	*	ns
	203	565	73.3	2853	-34.9	**2114**	**	*	ns
	217	236	72.0	731	13.2	**842**	*	*	ns

(the earliest the vaccine could have an effect is from day 56)

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

Antibody titres in the unvaccinated control ewes remained at background levels close to zero throughout the trial (Fig 4). In contrast, group mean titres in the Previously Vaccinated group fluctuated around the 10,000 mark from the trial start until their second boost given at marking time. Meanwhile, titres in the First Vaccinated ewes rose slowly to reach a mean of about 2,400 at marking time. Thereafter both vaccinated groups responded to each vaccine boost with a temporary spike in titre observed at the next sampling. Mean titres in the Previously Vaccinated ewes always exceeded those of the First Vaccinates by at least two-fold (Fig 4).

Total and *Haemonchus* specific group mean faecal egg counts were quite similar and followed very similar patterns in all groups, reflecting the fact that *Haemonchus* was usually the dominant gastrointestinal nematode genus infecting the ewes (Fig 4, Table A3).

Mean Control egg counts were negligible at the start of the trial, rising slowly to about 1,400 during November and December, before increasing sharply to peak at 6,499 epg during January (Fig 4, Table 1). Control counts fell away rapidly during February (Fig 4, Table 1), probably as a consequence of the low pasture infectivity brought about by the January drought (Fig 1), because the onset of rain in March resulted in another surge of egg output (Fig 4, Table 1) later in that month.

Mean *Haemonchus* egg counts were nearly always lower in both vaccinated groups compared to the controls. This was especially in the case of the Previously Vaccinated group where the difference was highly significant, statistically, at every sampling during lactation bar one (Table 1). Barbervax did provide some limited protection to the First Vaccine group, but this was only statistically significant on three occasions during the trial (Table 1) and the egg counts of this group were usually significantly higher than those of the Previously Vaccinated ewes.

These kinetics and group differences in *Haemonchus* specific egg counts were inversely reflected in the degree of anaemia and precautionary drenching (Fig 5). Thus, peak egg counts in January coincided with the lowest blood haemoglobin concentrations and when most precautionary drenching was required (Fig 5) and the Previously Infected group was significantly ($P<0.05$) less anaemic than the controls around this time (Table 4).

Fig 4. Kinetics of interventions, group mean total egg counts, *Haemonchus* specific egg counts and the anti vaccine antibody response.

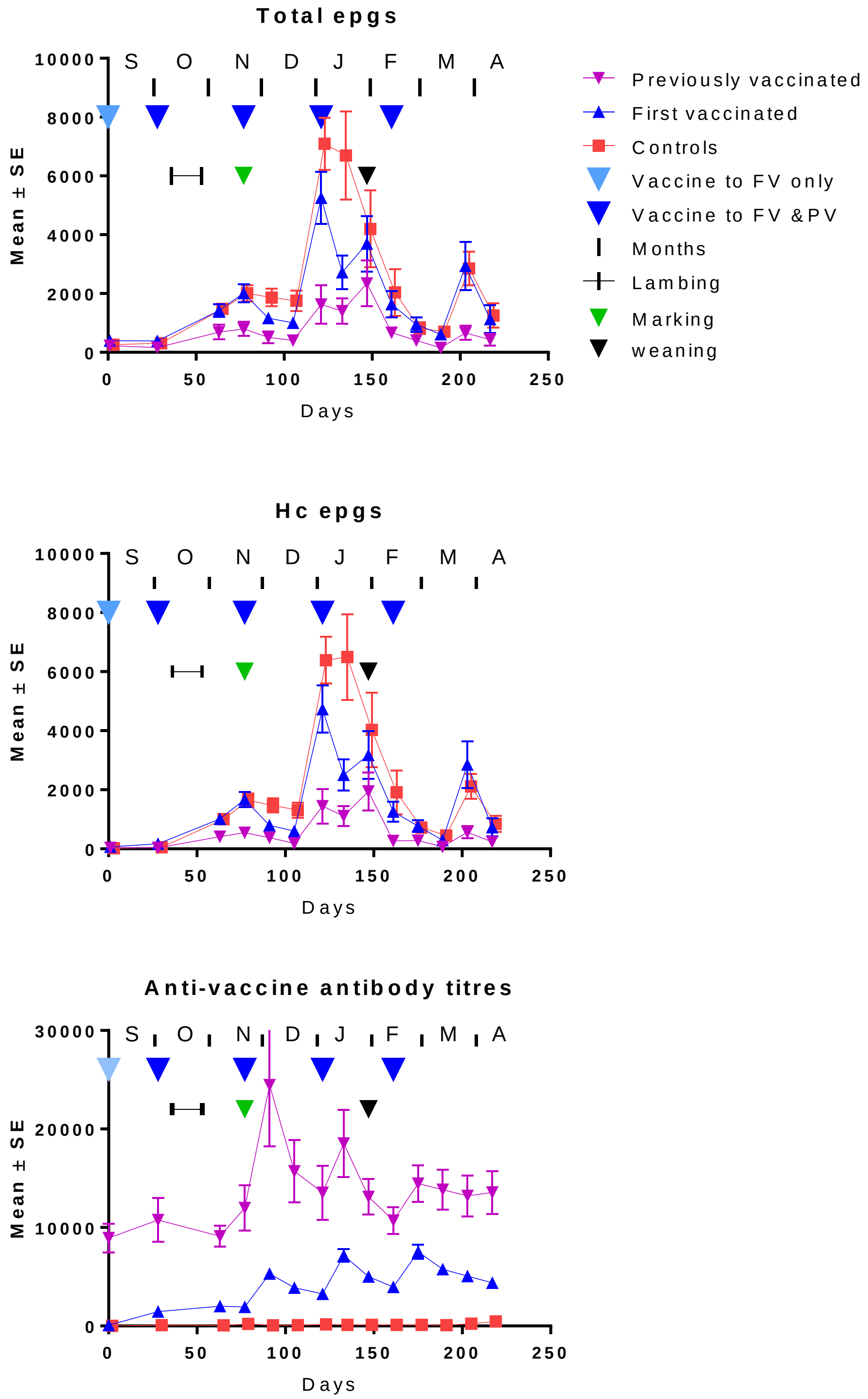


Fig 5. Kinetics of the *Haemonchus* specific egg counts in relation to blood haemoglobin concentrations and to precautionary drenching.

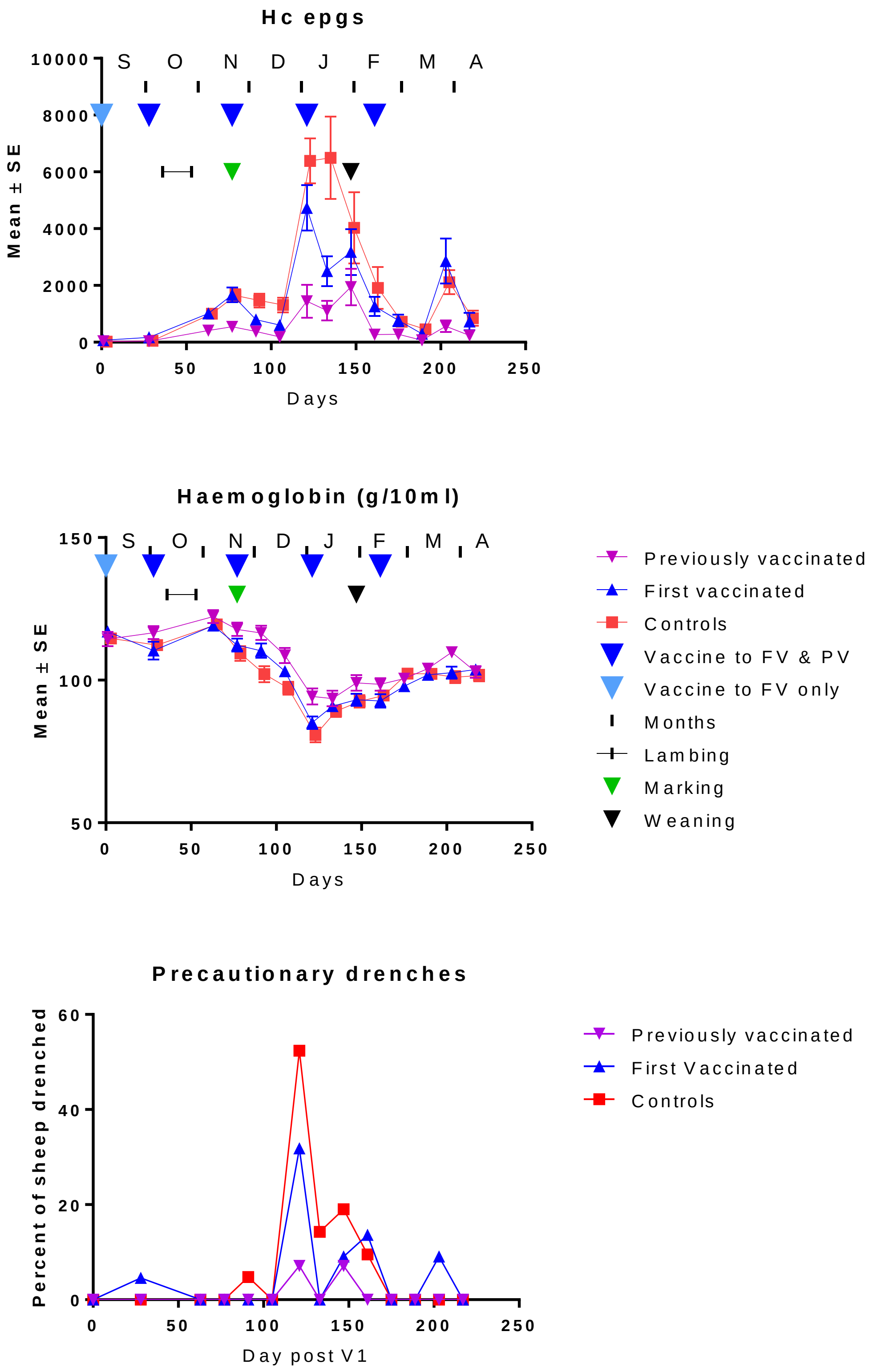


Table 2. Mean blood haemoglobin concentration at each sampling (g/10ml)

Event	**Day**	**PV**	**FV**	**Cont**	**P value by ANOVA**		
		Mean	Mean	Mean	**PV vs FV**	**PV vs Cont**	**FV vs Cont**
V1	**0**	114.6	117.0	115.2	ns	ns	ns
V2	**28**	116.4	109.9	112.3	ns	ns	ns
	63	122.4	119.6	120.1	ns	ns	ns
V3	**77**	117.4	112.4	108.6	ns	ns	ns
	91	116.5	110.3	100.8	ns	ns	ns
	105	107.1	102.8	95.9	ns	<0.01	ns
V4	**121**	92.7	84.1	77.8	<0.05	<0.01	ns
	133	91.5	90.5	82.2	ns	<0.001	ns
weaning	**147**	97.4	92.7	92.3	ns	<0.05	ns
V5	**161**	96.3	90.9	94.5	ns	ns	ns
	175	99.5	97.6	102.9	ns	ns	ns
	189	104.3	101.5	101.9	ns	ns	ns
	203	108.8	102.1	99.3	ns	<0.05	ns
	217	103.5	102.7	100.2	ns	ns	ns

Table 3. Number of precautionary drenches given per group.

	Number of sheep treated			Fishers exact test		
	PV	**FV**	**Controls**	**PV vs FV**	**PV vs Cont**	**FV vs Cont**
No of sheep per group	**14**	**22**	**21**			
Day						
28	0	1	0			
91	0	0	1			
121	1	7	11	ns	<0.05	ns
133	0	0	3			
147	1	2	4		ns	
161	0	3	2			
203	0	2	0			
Total	**2**	**15**	**21**	<0.01	<0.001	<0.01

d. Bodyweights.

The ewes were weighed pre-lambing, at the time the First Vaccinates received their first boost, at marking, at weaning and at the end of the trial. Group mean bodyweights are presented in Fig 6 below. No significant differences (p> 0.05 by ANOVA) were detected between the groups at any stage of trial.

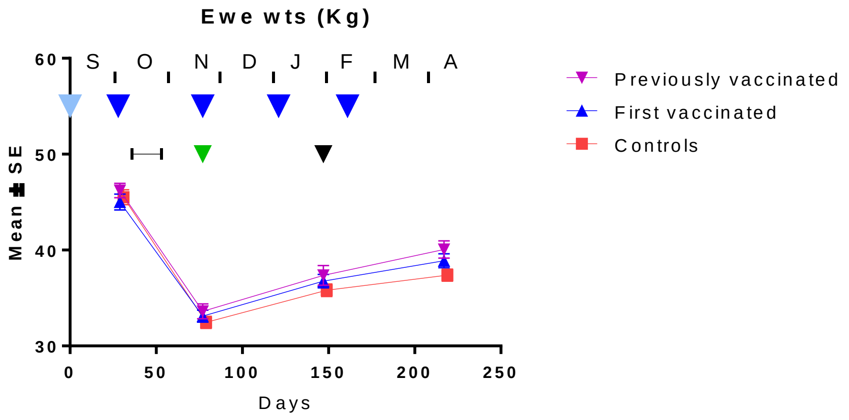


Fig 6. Ewe weights during the course of the trial

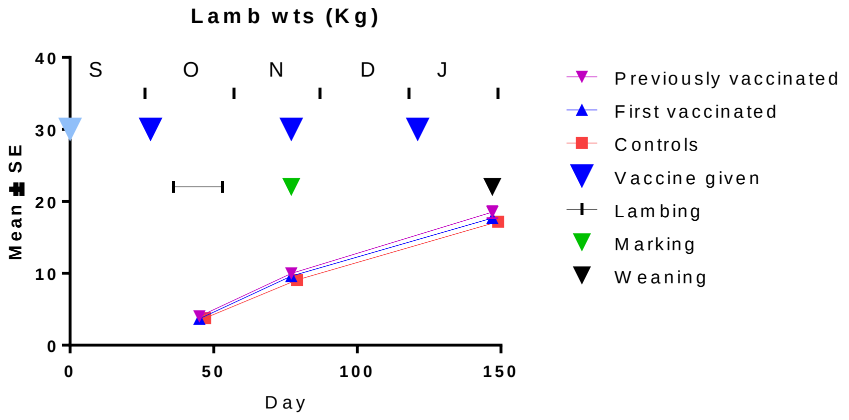


Fig 7. Lamb weights at birth, marking and weaning.

The lambs were weighed at birth, marking and weaning. Group mean liveweights are plotted in Fig 7. No significant differences ($p > 0.05$ by ANOVA) were detected between the groups at any stage of trial.

It would appear that the benefit of improved *Haemonchus* control in the vaccinated ewes, did not translate into better lamb growth rates, by means of improved milk production. It should be borne in mind however, that nearly all the Control ewes required a precautionary anthelmintic during lactation which is likely to have confounded the true result.

e. Discussion/Conclusions

In this study Barbervax was evaluated in Merino ewes around lambing time, during lactation and after weaning. One group had received two courses of Barbervax as lambs and yearlings in previous summers and a second was immunised for the first time, their initial injection being given some 6-8 weeks before lambing. The faecal egg counts, blood haemoglobin concentrations and serology of these vaccinates were compared with those of age matched unvaccinated controls. For simplicity, and to ensure equal exposure of the groups to the natural challenge infection, all sheep grazed together throughout the trial. To avoid the complication of the lamb egg output adding to the *Haemonchus* epidemiology, their lambs were treated regularly with anthelmintic.

As it turned out the summer of 2013-2014 was exceptionally dry in northern NSW, with hardly any rain falling in January, for example. The consequence was that pasture larval infectivity was very low in mid November and early February as demonstrated by the tracer sheep (Fig 1). Fortunately however, the natural larval challenge was still adequate to provide *Haemonchus* egg counts and associated anaemia in the controls sufficient to make comparison with the vaccinates scientifically valid.

The results clearly showed that ewes which had been vaccinated in an earlier season were significantly protected against the challenge as measured by reduced *Haemonchus* egg output and blood loss compared to the unvaccinated control ewes. These differences were evident during lactation as well as post weaning.

Interestingly, these Previously Vaccinated sheep were much better protected than ewes vaccinated for the first time, despite the fact that the latter received an additional immunisation before lambing. The First Vaccinated group developed much weaker antibody titres than the Previously Vaccinated animals, such that they were barely protected relative to the Controls.

Perhaps it takes more nutritional resource for a naive sheep to mount a primary immune response, compared to that required for a secondary response in an animal primed by a previous course of vaccine. In heavily pregnant and or lactating ewes protein and energy resources are particularly limited, which is likely to be responsible for the well-known periparturient relaxation of immunity to gastro-intestinal nematode parasites in sheep (e.g. Kahn, L. http://www.wormboss.com.au/news/articles/nonchemical-management/why-are-lambing-ewes-susceptible-to-worm-infection.php).

Whatever the mechanism, the observation that the Previously Vaccinated ewes had greatly reduced egg counts during lactation was important because the egg output of ewes at this time is considered to be the main source of infection for the next generation of lambs. Curbing ewe egg output during lactation by use of Barbervax should retard and reduce the build up of pasture infectivity which usually peaks in late summer.

It was interesting to note that the Previously Vaccinated ewes possessed substantial anti-vaccine antibody titres even before they were given their first boost in the current trial. The same observation was made with previously vaccinated yearlings in the spring of 2012. It was not clear whether these antibodies would have been protective in either trial, though correlations derived from pen trials conducted at Moredun and described in the first registration dossier suggest that they should have been. Nor is it known how long such titres and potentially protection would have persisted, or whether the first boost to Previously Vaccinated ewes could have been delayed to a subsequent muster, e.g. marking or weaning. If so, producers could use fewer doses of vaccine to maintain *Haemonchus* control. These important practical questions can only be addressed by running further trials.

No adverse effects of the vaccine were observed. Vaccination did not lead to statistically superior weight gains in the ewes or their lambs. However, this data was confounded because almost all the control ewes also required anthelmintic support, without which some may have succumbed to fatal Haemonchosis.

Appendix 1 Tabulated and raw data.

Table A.1

Blood Haemoglobin concentrations for trial ewes(g/10ml)

= treated because Hb=<7.5 g/10ml   = treated because epg>10,000.

Ewe no	Group	Lamb raised	Day after V1: 0	28	63	77	91	105	121	133	147	161	175	189	203	217
2011A 0098	PV	y	126	123	130	125	127	111	80	93	100	98	103	110	110	107
2011A 0118	PV	y	129	123	131	119	124	119	105	101	105	94	97	104	116	107
2011A 0144	PV	y	98	108	108	105	107	100	92	86	90	97	94	99	107	102
2011A 0155	PV	y	124	123	128	127	122	116	98	94	112	108	109	110	120	107
2011A	PV	y	110	106	125	117	117	107	91	90	113	96	99	111	110	99

7113																
2011A																
7115	PV	y	114	122	107	107	120	107	90	98	99	91	90	103	102	98
2011A																
7116	PV	y	109	117	123	125	118	116	99	96	100	95	107	113	110	114
2011A																
7126	PV	n	119	126	122	124	118	123	102	111	115	107	109	97	118	105
2011A																
7127	PV	y	103	112	120	114	111	93	75	89	95	96	103	95	101	98
2011A																
7137	PV	n	107	110	121	117	116	116	109	102	106	106	107	108	115	92
2011A																
7163	PV	y	126	136	145	134	135	125	116	115	115	114	105	108	118	115
2011A																
7165	PV	y	105	102	113	113	104	90	86	84	86	87	95	97	97	89
2011A																
7168	PV	y	126	118	115	96	92	100	90	86	88	111	110	116	114	98
2011A	PV	y	117	121	119	122	116	113	100	89	91	95	89	106	114	113

7178																
2011A 7180	PV	y	101	106	123	117	112	93	80	78	83	82	87	88	97	93
2011A 7186	PV	y	116	113	127	122	126	110	96	80	86	99	105	100	107	109
2011A 7075	FV	y	115	124	126	123	123	101	93	96	86	75	94	98	100	102
2011A 7102	FV	y	116	107	129	119	114	118	88	80	80	76	89	102	100	99
2011A 7125	FV	y	129	117	123	114	112	98	79	93	105	104	100	109	109	113
2011A 7144	FV	y	115	108	126	116	104	106	74	104	112	99	104	110	111	117
2011A 7145	FV	y	117	103	119	138	128	106	72	88	89	90	100	95	85	105
2011A 7172	FV	y	118	124	124	119	121	110	94	89	78	74	101	102	111	97
2011A	FV	y	118	115	120	97	99	98	72	94	113	110	113	97	90	103

7179																
2011A 7187	FV	y	128	117	134	119	114	109	81	86	78	110	104	110	111	103
2011A 7189	FV	y	117	116	128	126	130	106	80	91	88	92	92	101	109	109
2011A 7387	FV	y	119	117	115	102	106	92	72	84	95	95	102	97	102	95
2011A 7396	FV	n	132	121	110	110	110	111	109	96	104	100	106	111	114	124
2011A 7407	FV	y	115	114	127	115	112	112	86	86	90	88	92	94	94	104
2011A 7425	FV	y	120	115	130	122	127	106	82	104	106	90	101	106	115	105
2011A 7430	FV	n	104	*	*	*	*	*	*	*	*	*	*	*	*	*
2011A 7446	FV	y	111	105	105	100	90	97	94	92	79	107	105	104	88	101
2011A	FV	y	125	113	115	101	104	91	87	93	91	96	93	76	94	97

7453																
2011A																
7463	FV	n	120	49	117	108	127	112	95	85	85	70	98	104	113	104
2011A																
7465	FV	y	102	103	100	96	95	91	84	80	85	85	84	92	104	95
2011A																
7624	FV	y	105	102	114	104	104	95	88	92	95	92	97	105	92	99
2011A																
7656	FV	y	120	118	123	113	105	114	101	104	99	109	97	108	118	116
2011A																
7658	FV	y	136	128	123	127	121	121	97	91	101	100	105	116	119	115
2011A																
7665	FV	y	127	114	119	101	107	98	91	89	100	96	101	102	104	100
2011A																
7667	FV	y	104	96	103	94	85	82	67	82	90	86	85	97	91	83
2011A																
7696	FV	y	102	102	109	116	93	95	75	91	92	91	88	97	89	98
2011A	FV	y	112	121	120	115	115	107	82	90	94	90	99	113	100	105

7702																
2011A																
7106	Cont	n	124	114	114	111	101	105	95	105	102	91	101	105	100	107
2011A																
7112	Cont	y	106	99	107	113	108	86	84	78	68	90	103	96	96	98
2011A																
7129	Cont	y	128	111	129	110	102	96	76	94	90	87	92	104	116	106
2011A																
7157	Cont	n	101	108	113	110	113	108	106	98	85	80	83	94	105	102
2011A																
7183	Cont	y	133	130	140	130	117	110	86	94	81	86	107	119	125	113
2011A																
7185	Cont	y	116	114	122	98	94	90	80	60	90	93	100	95	92	106
2011A																
7203	Cont	y	98	110	115	112	112	110	72	99	105	105	105	107	110	109
2011A																
7384	Cont	y	125	123	124	117	107	101	85	105	112	105	111	103	97	97
2011A	Cont	n	115	120	127	127	125	119	94	98	91	99	115	112	125	114

7390																
2011A																
7400	Cont	n	107	107	112	105	99	88	91	84	84	98	106	104	97	114
2011A																
7414	Cont	y	109	111	117	122	117	101	75	92	105	93	101	101	88	88
2011A																
7442	Cont	y	116	116	123	113	101	84	79	91	97	98	101	97	106	105
2011A																
7447	Cont	y	115	107	122	110	96	98	71	93	102	92	94	107	100	88
2011A																
7452	Cont	y	115	113	120	113	111	96	75	93	96	95	103	96	96	102
2011A																
7454	Cont	y	126	121	124	117	104	102	90	84	80	85	100	98	104	114
2011A																
7458	Cont	y	109	117	123	121	115	101	82	75	98	106	106	103	91	87
2011A																
7488	Cont	y	122	119	135	128	119	115	109	86	78	70	90	104	115	112
2011A	Cont	y	111	116	128	105	96	83	64	95	109	105	106	105	101	108

7497 2011A																
7498 2011A	Cont	y	109	111	100	94	87	88	71	89	92	93	96	100	99	100
7626 2011A	Cont	y	111	97	105	89	84	83	73	91	99	102	110	97	83	89
7632 2011A	Cont	y	104	97	116	78	63	93	79	75	102	101	109	102	100	96
7650 2011A	Cont	y	113	111	112	92	86	82	68	86	86	90	89	89	93	98
7662 2011A	Cont	y	116	108	124	116	117	103	74	87	89	93	98	96	96	103
7678 2011A	Cont	y	122	106	116	94	81	80	55	91	94	96	105	95	90	73
7706	Cont	y	115	121	121	109	99	108	86	85	78	113	127	127	102	112

Table A.2 Faecal worm egg counts (eggs/g faeces) for trial ewes.

Precautionary drenches during the trial : Hb <u><</u>75mg/mL yellow background, or FEC<u>></u>10000 pink background.

Ewe no	Group	Lamb raised	Day after V1 0	28	63	77	91	105	121	133	147	161	175	189	203	217
2011A 0098	Prev V	y	400	0	400	500	700	200	700	600	500	300	200	200	300	300
2011A 0118	Prev V	y	0	0	300	500	800	0	600	1600	3800	1700	1100	600	200	100
2011A 0144	Prev V	y	200	600	800	700	400	500	1500	1400	1600	800	600	100	500	700
2011A 0155	Prev V	y	300	400	800	800	400	400	400	800	1300	800	600	400	0	100
2011A 7113	Prev V	y	0	0	100	0	0	0	0	0	0	200	200	0	700	100
2011A	Prev V	y	400	100	0	0	0	0	0	600	1900	700	1300	600	1300	0

7115																
2011A																
7116	Prev V	y	0	0	800	500	100	300	700	1500	1800	600	0	100	400	700
2011A																
7126	Prev V	n	400	100	600	200	500	0	100	100	1900	800	1700	800	3600	1300
2011A																
7127	Prev V	y	300	500	1400	2000	700	1300	3400	0	100	200	100	0	3000	400
2011A																
7137	Prev V	n	300	200	100	2400	100	300	0	0	300	200	400	100	100	200
2011A																
7163	Prev V	y	0	0	100	300	300	0	0	0	0	100	200	0	0	0
2011A																
7165	Prev V	y	0	100	400	2500	100	200	1400	1500	2200	1900	0	0	100	0
2011A																
7168	Prev V	y	1300	300	3600	2700	3000	1900	8400	5100	10500	0	0	100	2200	2800
2011A																
7178	Prev V	y	0	0	200	200	100	0	0	300	1300	500	700	0	0	0
2011A	Prev V	y	200	300	700	200	400	300	400	700	1300	800	600	0	0	100

7180																
2011A																
7186	Prev V	y	100	0	0	200	200	600	5300	4200	6500	100	0	0	600	700
2011A																
7075	First V	y	500	0	1000	500	1500	1500	1500	1500	7000	3000	0	0	400	500
2011A																
7102	First V	y	300	0	800	500	500	600	3200	4600	9100	800	1800	200	500	200
2011A																
7125	First V	y	100	300	1500	1300	1300	200	10000	0	600	0	500	500	1900	100
2011A																
7144	First V	y	100	400	2300	2700	3400	1500	8000	0	200	300	0	200	1000	400
2011A																
7145	First V	y	0	0	100	100	200	1800	4800	0	400	100	200	2000	10600	0
2011A																
7172	First V	y	600	700	800	700	500	700	1200	3100	5300	5000	0	100	600	1300
2011A																
7179	First V	y	1300	900	3800	5100	1400	1800	17100	0	1200	400	1300	1400	14400	0
2011A	First V	y	2100	200	2900	2900	2300	3000	9200	8600	17700	0	0	500	4000	1400

7187																
2011A																
7189	First V	y	0	0	500	600	1200	1100	3300	2200	6200	3100	1800	100	400	200
2011A																
7387	First V	y	100	600	2000	4300	1800	3400	11000	0	300	600	600	1000	6200	9800
2011A																
7396	First V	n	300	200	500	500	1200	300	1600	2000	3600	1500	1300	1200	4800	600
2011A																
7407	First V	y	0	200	300	300	100	200	400	800	1200	1200	700	0	400	700
2011A																
7425	First V	y	0	0	1500	900	700	500	4500	1600	3600	2000	200	0	0	0
2011A																
7430	First V	n	400	500	dead											
2011A																
7446	First V	y	400	300	2200	4400	2900	600	4100	4900	11400	0	0	0	100	200
2011A																
7453	First V	y	100	200	1300	1800	0	375	2200	1000	1600	100	2000	100	500	100
2011A	First V	n	100	200	0	0	100	0	3500	1700	1800	1900	0	100	3600	1700

7463																
2011A																
7465	First V	y	500	500	800	2200	600	1000	3800	1500	2700	2200	1000	600	800	600
2011A																
7624	First V	y	400	500	200	1000	700	500	400	300	1200	1000	500	1200	1000	1100
2011A																
7656	First V	y	300	1300	2700	2300	1800	300	3800	2400	4800	4100	4700	1100	2800	1100
2011A																
7658	First V	y	200	500	1000	1900	800	200	7600	5000	3900	7400	500	600	1400	600
2011A																
7665	First V	y	700	1000	2900	3300	1100	842	1700	2100	2000	1000	1700	2500	3100	1400
2011A																
7667	First V	y	600	600	400	1000	2000	1300	8100	0	200	0	0	500	8300	700
2011A																
7696	First V	y	400	200	1000	3100	0	100	7800	0	0	300	300	200	5200	1700
2011A																
7702	First V	y	0	100	1300	3400	700	600	1900	1200	800	100	1100	600	1100	400
2011A	Control	n	200	0	900	300	300	0	0	0	2900	2700	4900	1600	1900	300

7106																
2011A																
7112	Control	y	0	500	2100	2500	1100	700	6300	9800	17900	0	0	200	600	100
2011A																
7129	Control	y	200	200	1000	1200	1400	800	3300	1700	7000	6700	1900	900	2100	1900
2011A																
7157	Control	n	0	100	0	0	100	0	1600	2900	8600	4900	8800	200	1200	200
2011A																
7183	Control	y	200	200	600	500	1200	1000	3500	1700	9000	12300	0	0	500	500
2011A																
7185	Control	y	400	300	1400	3200	3500	5900	8400	15100	0	0	0	1100	2100	500
2011A																
7203	Control	y	0	100	1400	900	1200	600	5100	0	400	300	700	0	100	0
2011A																
7384	Control	y	200	500	2100	2600	2600	4200	11000	0	2300	1600	2000	2700	8700	5300
2011A																
7390	Control	n	0	0	0	300	0	300	4200	5200	10000	0	0	800	2600	1100
2011A	Control	n	1600	1700	1200	1100	800	200	2200	3800	11900	0	0	600	6500	800

7400																
2011A																
7414	Control	y	0	0	700	800	1500	1600	11000	0	700	600	1000	600	5200	500
2011A																
7442	Control	y	600	600	800	2200	1400	2700	3100	2500	2900	2100	2900	500	1800	1200
2011A																
7447	Control	y	0	100	1900	1200	600	353	5300	0	200	300	1000	0	200	300
2011A																
7452	Control	y	100	200	1500	2800	3700	800	5100	0	300	100	500	300	4100	500
2011A																
7454	Control	y	0	0	200	100	700	300	2400	2900	5900	3700	2000	300	3400	700
2011A																
7458	Control	y	0	300	600	1300	1300	1200	3300	12600	0	0	0	1300	5100	2600
2011A																
7488	Control	y	300	300	1300	1400	600	100	4600	5100	9300	8700	0	100	0	200
2011A																
7497	Control	y	500	300	2200	1800	1600	2500	12400	0	300	200	600	0	1700	300
2011A	Control	y	100	200	1700	3300	4800	1600	9900	0	1300	900	1100	300	1500	500

7498																
2011A 7626	Control	y	1000	600	3600	3600	2100	3200	10200	0	400	100	700	2000	8000	900
2011A 7632	Control	y	100	700	1100	2100	1000	0	5800	8000	0	0	100	200	1800	1300
2011A 7650	Control	y	300	500	2000	2000	600	700	5300	0	400	200	200	300	1600	600
2011A 7662	Control	y	500	400	1900	400	1100	700	5800	0	900	700	900	2600	3100	700
2011A 7678	Control	y	300	400	1900	5400	5500	4100	19200	0	400	200	300	1300	7600	7600
2011A 7706	Control	y	800	100	1000	2900	1600	2000	8100	7600	15900	0	0	0	800	200

Table A.3 Larval differentiation results from ewe trial copro-cultures

Group	Date	Haem	Trich.	Tela	Oesoph	Other	Total	% Haem
PV	04/09/2013	18	77	5	0	0	100	18%
FV	04/09/2013	18	74	8	0	0	100	18%
Cont	04/09/2013	9	87	4	0	0	100	9%
PV	02/10/2013	21	72	7	0	0	100	21%
FV	02/10/2013	44	48	8	0	0	100	44%
Cont	02/10/2013	18	70	12	0	0	100	18%
Tracers	02/10/2013	57	37	6	0	0	100	57%
PV	06/11/2013	60	36	4	0	0	100	60%
FV	06/11/2013	71	23	6	0	0	100	71%
Cont	06/11/2013	68	29	3	0	0	100	68%
Tracers	06/11/2013	7	0	0	0	0	7	?
PV	20/11/2013	69	26	5	0	0	100	69%
FV	20/11/2013	83	15	2	0	0	100	83%
Cont	20/11/2013	82	14	4	0	0	100	82%
Tracers	20/11/2013	0	9	0	0	0	9	?
PV	18/12/2013	46	44	10	0	0	100	46%
FV	18/12/2013	60	27	12	1	0	100	60%
Cont	18/12/2013	75	21	3	1	0	100	75%
Tracers	18/12/2013	29	8	4	2	0	43	67%
PV	03/01/2014	89	7	4	0	0	100	89%
FV	03/01/2014	89	11	0	0	0	100	89%
Cont	03/01/2014	90	9	1	0	0	100	90%
Tracers	03/01/2014	97	3	0	0	0	100	97%
PV	15/01/2014	79	16	5	0	0	100	79%
FV	15/01/2014	92	7	1	0	0	100	92%

Group	Date	Haem	Trich.	Tela	Oesoph	Other	Total	% Haem
Cont	15/01/2014	97	2	0	0	0	99	98%
Tracers	15/01/2014	0	0	0	0	0	no larvae	?
PV	29/01/2014	83	15	2	0	0	100	83%
FV	29/01/2014	86	10	4	0	0	100	86%
Cont	29/01/2014	96	1	3	0	0	100	96%
Tracers	29/01/2014	97	3	0	0	0	100	97%
PV	12/02/2014	41	44	15	0	0	100	41%
FV	12/02/2014	77	19	4	0	0	100	77%
Cont	12/02/2014	94	5	1	0	0	100	94%
Tracers	12/02/2014	93	7	0	0	0	100	93%
PV	26/02/2014	70	26	4	0	0	100	70%
FV	26/02/2014	82	18	0	0	0	100	82%
Cont	26/02/2014	93	7	0	0	0	100	93%
Tracers	26/02/2014	0	0	0	0	0	no larvae	?
PV	12/03/2014	47	49	4	0	0	100	47%
FV	12/03/2014	49	46	5	0	0	100	49%
Cont	12/03/2014	65	30	5	0	0	100	65%
Tracers	12/03/2014	17	0	0	0	0	17	100%
PV	26/03/2014	85	15	0	0	0	100	85%
FV	26/03/2014	87	12	1	0	0	100	87%
Cont	26/03/2014	74	22	4	0	0	100	74%
Tracers	26/03/2014	86	10	4	0	0	100	86%
PV	09/04/2014	55	37	8	0	0	100	55%
FV	09/04/2014	65	33	2	0	0	100	65%
Cont	09/04/2014	61	37	2	0	0	100	61%
Tracers	09/04/2014	67	31	2	0	0	100	67%

Table A.4 Antibody titres

			Days after V1													
Ewe	**Grp**	**Lamb**	**0**	**28**	**63**	**77**	**91**	**105**	**121**	**133**	**147**	**161**	**175**	**189**	**203**	**217**
2011A 0098	PV	y	5452	5873	6111	6988	9866	11460	9385	10788	8943	7658	9684	10,124	9,618	9,127
2011A 0118	PV	y	22399	35517	19170	37575	88193	50794	25269	40973	22302	20753	32500	31,464	35,069	35,844
2011A 0144	PV	y	4436	4401	6764	6912	12984	9974	9029	14074	10945	8650	9865	9,061	8,423	8,456
2011A 0155	PV	y	19161	20595	13675	16515	54527	24488	44776	49223	26435	21554	21468	28,134	22,848	23,754
2011A 7113	PV	y	10121	11927	10752	12840	22830	17081	15146	18262	15568	12410	12895	13,446	13,494	14,351
2011A 7115	PV	y	7307	7652	7622	8618	17511	11838	9678	15563	11996	8892	15090	13,442	11,454	11,317
2011A 7116	PV	y	10379	11045	10811	12196	31201	25103	13073	24406	24352	13446	18742	18,121	16,155	17,987

2011A 7126	PV	n	5981	6405	6734	7320	10345	8303	8253	10060	8013	6773	9721	9,267	8,403	8,380
2011A 7127	PV	y	4593	5049	4419	4907	6035	5103	5104	6577	5605	4978	6622	5,689	6,093	5,664
2011A 7137	PV	n	5729	6675	6717	6731	9620	7992	6927	9371	7378	6989	8144	7,319	7,844	218
2011A 7163	PV	y	6183	6977	6437	7094	8665	8116	7622	10032	8162	6942	12004	10,312	9,075	8,539
2011A 7165	PV	y	5881	6676	6687	7823	11747	8598	8460	11043	8029	7112	9777	8,424	8,097	7,875
2011A 7168	PV	y	7166	8533	8003	9374	10765	10141	9954	8863	8321	8212	11282	11,088	10,350	10,260
2011A 7178	PV	y	6133	6883	8252	8861	17122	11121	10330	15681	10491	9143	11582	9,982	9,186	10,193
2011A 7180	PV	y	10207	13511	12892	21729	43123	19405	14706	26125	15487	13527	21700	17,118	18,094	18,820
2011A 7186	PV	y	5699	5929	5969	6525	7877	6831	6953	7788	6889	6540	9089	7,276	7,150	7,560

2011A 7075	FV	y	0	76	112	53	2483	1055	810	4736	2456	1047	3107	1,427	1,498	907
2011A 7102	FV	y	0	117	3807	3127	8528	7288	5390	7547	5800	4593	10759	8,049	6,303	5,305
2011A 7125	FV	y	9	821	2426	1952	6281	4655	4613	7440	5917	5083	9414	7,309	6,430	5,746
2011A 7144	FV	y	45	2560	2629	2413	6092	4446	3206	7517	5639	4717	6604	5,715	5,629	5,561
2011A 7145	FV	y	0	1185	422	273	4183	2477	2301	5600	4427	3115	7231	5,613	4,618	3,799
2011A 7172	FV	y	348	345	372	65	2532	1276	998	3971	1886	979	3704	2,174	2,257	1,500
2011A 7179	FV	y	177	617	874	412	1641	1164	1045	3742	1867	634	3693	1,844	1,606	817
2011A 7187	FV	y	28	209	534	192	3002	2145	1275	4268	2143	898	4482	3,163	3,110	2,169

2011A 7189	FV	y	0	611	338	162	4541	3134	2325	6046	4078	2585	9298	6,162	6,369	5,440
2011A 7387	FV	y	6	902	82	106	1600	526	218	4283	2357	909	4116	2,404	2,332	1,478
2011A 7396	FV	n	492	970	4346	4687	18118	12777	6549	13869	9264	7151	10920	10,978	8,539	8,690
2011A 7407	FV	y	42	3500	3431	3575	6325	4985	4345	7129	5484	4809	6609	5,170	4,847	4,601
2011A 7425	FV	y	696	2485	1779	3038	6071	4406	3693	8775	6001	4614	7001	6,446	5,268	4,483
2011A 7430	FV	n	94													
2011A 7446	FV	y	254	3065	4533	4659	7430	5230	4522	8057	5837	4988	7432	6,470	5,650	5,496
2011A 7453	FV	y	1	168	1448	1335	5960	4693	4289	9269	6215	5438	13458	9,613	7,173	6,192
2011A 7463	FV	n	14	1380	12437	10535	43367	14925	10308	30200	17154	11723	13841	12,911	12,814	11,575

2011A 7465	FV	y	1	1405	2117	2426	6064	4945	4703	8088	6344	5408	7900	6,559	6,346	5,442
2011A 7624	FV	y	385	2654	4261	3463	10606	7167	6043	17553	9860	7293	13989	10,095	7,464	6,949
2011A 7656	FV	y	1	3933	3954	3227	5698	4818	4199	7145	5515	4714	6530	5,684	4,895	4,587
2011A 7658	FV	y	0	266	2076	2641	4742	3816	2745	5772	4768	4058	5872	5,239	4,547	4,170
2011A 7665	FV	y	0	983	1691	2052	7718	5621	5005	8164	6620	6329	8320	6,915	6,659	5,801
2011A 7667	FV	y	0	2957	2325	3158	4421	3375	2977	5577	4789	4352	5767	4,953	4,570	3,640
2011A 7696	FV	y	3	1262	783	741	4871	3393	3060	5923	5194	4433	6101	5,454	5,016	4,488
2011A 7702	FV	y	1	1963	4042	3427	6020	4695	3974	10932	7238	6296	14323	10,169	8,941	7,700
2011A 7106	Cont	n	0	1	341	0	0	0	9	0	1	1	237	22	88	10

2011A 7112	Cont	y	7	1	0	11	1	1	139	3	8	66	0	3	2	28
2011A 7129	Cont	y	1	2	5	34	4	1	6	68	2	6	15	93	2,066	737
2011A 7157	Cont	n	5	3	18	45	33	61	95	284	516	332	331	279	214	290
2011A 7183	Cont	y	1	11	0	8	10	37	54	98	70	51	28	12	18	47
2011A 7185	Cont	y	0	1	0	1	4	2	38	4	400	9	4	29	9	31
2011A 7203	Cont	y	0	0	0	0	0	1	2	2	0	0	0	3	1	73
2011A 7384	Cont	y	32	53	25	19	21	16	38	4	1	1	4	9	8	5
2011A 7390	Cont	n	0	0	0	0	3	11	8	12	13	2	1	1	1	1

2011A 7400	Cont	n	0	1	0	6	45	12	47	39	31	98	60	30	262	7,249
2011A 7414	Cont	y	0	537	12	52	0	20	15	11	28	36	64	25	5	7
2011A 7442	Cont	y	14	53	52	65	271	160	448	895	480	617	518	283	189	215
2011A 7447	Cont	y	57	196	291	338	544	745	1079	452	162	261	370	768	1,736	1,701
2011A 7452	Cont	y	0	0	0	4	0	0	4	1	0	0	1	3	1	5
2011A 7454	Cont	y	0	0	0	0	0	0	18	2	0	17	15	13	2	9
2011A 7458	Cont	y	117	1	3	1	2	4	504	6	0	0	3	52	94	146
2011A 7488	Cont	y	0	0	0	29	2	4	11	3	0	13	2	1	7	0
2011A 7497	Cont	y	1	7	1	24	13	0	9	8	1	7	1	0	10	1

2011A 7498	Cont	y	4	34	8	0	21	26	30	26	16	83	62	58	175	370
2011A 7626	Cont	y	0	2	0	6	1	2	139	0	0	2	1	1	2	11
2011A 7632	Cont	y	2	46	0	4324	61	6	1	7	8	11	7	2	8	2
2011A 7650	Cont	y	1	18	3	0	5	11	19	17	9	67	39	5	12	3
2011A 7662	Cont	y	1	54	0	0	0	58	30	13	4	22	21	35	28	36
2011A 7678	Cont	y	277	938	602	154	221	781	1249	861	1071	1000	1144	576	758	366
2011A 7706	Cont	y	0	0	33	2	0	145	198	32	24	78	108	30	76	25

Table A.5. Bodyweights (Kg) of ewes which raised a lamb

Ewe no	Group	Lamb raised	Day after V1 29	77	147	217
2011A0098	Prev V	y	47	31.8	35.5	38.5
2011A0118	Prev V	y	47.5	35.6	35.5	41.5
2011A0144	Prev V	y	44	32.1	34.5	37.5
2011A0155	Prev V	y	48	33.2	36.5	38.5
2011A7113	Prev V	y	46	36.6	43	43.5
2011A7115	Prev V	y	48.5	40.2	44.5	45
2011A7116	Prev V	y	45.5	32.9	34.5	37.5
2011A7126	Prev V	n				
2011A7127	Prev V	y	41	30.6	35.5	40.5
2011A7137	Prev V	n				
2011A7163	Prev V	y	45.5	34.7	36.5	38
2011A7165	Prev V	y	47.5	31.8	41.5	41.5
2011A7168	Prev V	y	45.5	30.4	34.5	38
2011A7178	Prev V	y	53	37.8	43	47.5
2011A7180	Prev V	y	44.5	31.5	33.5	35.5
2011A7186	Prev V	y	43.5	31.3	34.5	38
2011A7075	First V	y	47.5	32.9	35.5	39
2011A7102	First V	y	40.5	31	32.5	35.5
2011A7125	First V	y	42.5	31.4	34.5	35.5
2011A7144	First V	y	41.5	31.7	36	36.5
2011A7145	First V	y	49	35.5	39.5	42
2011A7172	First V	y	51.5	33.9	41	44

2011A7179	First V	y	45.5	32.3	35.5	40
2011A7187	First V	y	41	29.5	33.5	34.5
2011A7189	First V	y	42	31.8	32.5	34.5
2011A7387	First V	y	51.5	37.4	42	41
2011A7396	First V	n				
2011A7407	First V	y	47	34.6	40.5	42
2011A7425	First V	y	47.5	34.6	37	40.5
2011A7446	First V	y	50	35	37	40.5
2011A7453	First V	y	41	28.9	31.5	34
2011A7463	First V	n				
2011A7465	First V	y	43.5	32.7	39.5	37.5
2011A7624	First V	y	37.5	30.9	33	34
2011A7656	First V	y	46.5	39.3	41.5	43.5
2011A7658	First V	y	43.5	33.2	36.5	39.5
2011A7665	First V	y	40.5	29	33	35.5
2011A7667	First V	y	49	39.8	41	44
2011A7696	First V	y	47	31.2	39	41.5
2011A7702	First V	y	44.5	32	36.5	40.5
2011A7106	Control	n				
2011A7112	Control	y	42	29.1	34.5	36
2011A7129	Control	y	43	30.3	33	34.5
2011A7157	Control	n	45.5	37.4	41	41.5
2011A7183	Control	y	48.5	33.7	37.5	40.5
2011A7185	Control	y	41.5	28.7	30	33.5
2011A7203	Control	y	40.5	30.1	34.5	34.5
2011A7384	Control	y	42	32.1	33.5	37.5
2011A7390	Control	n				

2011A7400	Control	n				
2011A7414	Control	y	49	34.3	37	38
2011A7442	Control	y	45	31	34	35.5
2011A7447	Control	y	49	32.1	38.5	34.5
2011A7452	Control	y	52	37.3	38.5	42.5
2011A7454	Control	y	43.5	35.4	36.5	37
2011A7458	Control	y	49.5	36	39	39
2011A7488	Control	y	49	34.3	38.5	41
2011A7497	Control	y	49.5	33.5	37	39
2011A7498	Control	y	48.5	35.6	38.5	40.5
2011A7626	Control	y	43.5	33.5	37.5	36.5
2011A7632	Control	y	42.5	30.3	36	37.5
2011A7650	Control	y	44	28.6	34	36
2011A7662	Control	y	50.5	31.8	37.5	40.5
2011A7678	Control	y	39.5	26	28	31.5
2011A7706	Control	y	43	33.2	33.5	35.5

Table A6

Weather Data from Australian Bureau of Meteorology

Armidale airport - monthly rainfall (mm)

2013-2014

S	O	N	D	J	F	M	A
25	*41.4*	*117*	*39.2*	*2.4*	*48.8*	*115.8*	*7.4*

Mean monthly rainfall (mm)

S	O	N	D	J	F	M	A
56.3	74.5	110.4	95.4	87.1	98.6	59.5	34.7

Appendix 6.2 Dundee ewe efficacy trial

VETERINARY HEALTH RESEARCH PTY LTD

STUDY REPORT

Study Title: A field study to evaluate the efficacy of an *Haemonchus* vaccine when administered to vaccinated and unvaccinated lactating ewes during times of high parasite challenge. New England district NSW, Australia.

Study No.: **MIHO2920** **Sponsor Study No.:** N/A

Version No.: **6** **FINAL** **Version Date:** 10 July 2014

Author: T. Dale

Sponsor:	Name:	Julie Fitzpatrick Moredun Group Director
	Address:	Moredun Institute The Moredun Group Pentlands Science Park Bush Loan Penicuik Midlothian Scotland, UK

VETERINARY HEALTH RESEARCH PTY LTD

STUDY REPORT

Sponsor Monitor & Representative:	Name:	David Smith
	Address:	The Moredun Group Pentlands Science Park Bush Loan Penicuik Midlothian Scotland, UK
Investigator:	Name:	Timothy Dale
	Quals.:	B. LISC
	Address	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: ______________________________

Timothy Dale, B.Li.Sc.

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
14 Aug 13	Study	Protocol V2 – 05 Aug 13	L. Pearson	14 Aug 13
01 Jul 14	Study	Study Report V4 – 17 Jun 14	L. Pearson	03 Jul 14

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 Pearson, DipRQA

Quality Assurance Manager

Date: ______________________________

1. OBJECTIVE

To confirm the field efficacy of a *Haemonchus* vaccine in previously vaccinated and unvaccinated peri-parturient ewes against *Haemonchus contortus*, when previously vaccinated, previously unvaccinated and control (unvaccinated) sheep are grazed together, in the northern New England region of New South Wales, Australia. Data from this study may be used to support product registration.

2. JUSTIFICATION

Commonly, 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 effective at reducing host anaemia and parasite egg output. This study aimed to investigate the efficacy in pregnant ewes (either vaccinated or not during the previous season) when given a course of immunizations starting before parturition.

3. COMPLIANCE

The study complied with the following national and international standards:

VICH GL9 Good Clinical Practice (issued June 2000)

4. TEST SITE(S)

Animal Phase:	**Laboratory Phase:**
Anonomous	Veterinary Health Research P/L
Dundee NSW 2370	Colin Blumer Animal Health Laboratory
Australia	Trevenna Road
	Armidale NSW 2350 Australia

5. STUDY DATES

Start date (animal phase): 22 AUG 13

Finish date (animal phase): 20 MAR 14

Finish date (laboratory phase): 20 May 14

6. STUDY DESIGN

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

b. Animal Model: The study used maiden Merino ewes on normal pre-lambing prepared paddocks which were contaminated by *Haemonchus contortus*.

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: Group 1 – all ewe hoggets which were vaccinated as part of the 2012/2013 yearling trial on this property. Seventeen (17) animals were available for inclusion within this group.

Groups 2 and 3 – Fifty (50) pregnant first lambing ewes were randomly selected from a larger flock, after excessively heavy or light ("outliers", up to ~10% of the flock) animals have been removed. All trial animals were weighed at selection on Day 0 and ranked from heaviest to lightest, sequentially blocked into blocks of two (2) animals and randomly allocated (draw from hat technique)

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 10.0, 2013). There were no statistical differences ($p<0.05$) between groups.

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

7. INVESTIGATIONAL VETERINARY PRODUCT

g. Investigational Veterinary Product:

Name:	BarberVax	Batch No.:	08
Composition:	*Haemonchus* antigen and saponin adjuvant	Expiry Date:	01 APR 15
Dose Level:	5µg antigen and 1mg saponin	WHP:	12 months

h. Source: WormVax Laboratory

Animal Health Laboratory

Dept of Agriculture and Food Western Australia

444 Albany Highway

Albany WA 6330

i. Storage: Refrigerated between 2 to 8^{o}C.

j. Safety: A MSDS was not provided. (See **Deviation #2**).

k. Assays: A Certificate of Analysis was provided (**Appendix 8**).

l. Drug Disposal: The disposal of all remaining IVP will be documented.

8. TREATMENT

Animals in Group 3 will be retained as untreated controls but individual animals in either Group 1, 2 or 3 will be treated with a short acting anthelmintic if:

- ***H.contortus*:** the egg count rises above 10,000 epg or if the blood haemoglobin concentration falls below 6.5 g/100mL
- **Other genera:** (indicated by larval differentiation): the individual animal egg count rises above 1500 epg, or scouring is evident. For a flock treatment, the upper limit is a mean of 1000 epg (though scouring is likely to be evident before this level is reached). See NTF #1.
- **Scouring**: Individuals will be treated if above an AWI Scour Score of 3.

a. Dose Calculation: Dose volume was 1mL IVP by subcutaneous injection. Anthelmintic treatment was calculated according to individual animal bodyweight using Day 0, 112 or Day 210 bodyweights.

b. Dose Preparation: The IVP was transported on ice bricks and gently shaken immediately prior to first treatment.

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

Table 1: Treatment Regime

Group	IVP Details	Dose Volume	Route	Treatment Day(s)	No. Animals
1	IVP	1.0 mL	Subcut.	Days 21, 70, 112, 154 and 196 Treat with an effective anthelmintic on Days 0 and 210	17
2	IVP	1.0 mL	Subcut.	Days 0, 21, 70, 112, 154 and 196 Treat with an effective anthelmintic on Days 0 and 210	25
3	Untreated controls	N/A	N/A	Treat with an effective anthelmintic on Days 0 and 210	25

Subcut. = Subcutaneous

All animals were treated using either a Simcro Vaccine Gun or NJ Phillips Vaccine Gun at a dose level of 1.0 mL subcutaneously. Study animals were observed at the time of treatment, no abnormalities were observed.

9. SCHEDULE OF EVENTS

Table 2: Schedule of Events

Study Day*	Date	Event
Pre-Trial	---	Obtained Animal Ethics Committee approval; Confirmed trial arrangements with Sponsor and Farmer that ran a previous study MIHO2898 which was run in northern portion of the NSW New England. All ewes were scanned in lamb (see **NTF #5**).
Day 0	22 AUG 13	All animals were weighed and allocated into Groups. Collected blood samples from animals in Groups 1, 2 and 3 for haemoglobin analysis, and faecal samples from Groups 1, 2 and 3 for FECs and group larval differentiation (see Deviation #1). Processed Groups 1, 2 and 3 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0}C. **Group 2 were vaccinated with 1.0 mL of IVP (V1).** All animals in Groups 1, 2 and 3 were treated with ZOLVIX to drench out animals. Commenced twice weekly observations.

Study Day*	Date	Event
Day 14	05 SEP 13	Collected blood samples from animals in Groups 1, 2 and 3 for haemoglobin analysis, and faecal samples from Groups 1, 2 and 3 for FECs and group larval differentiation (see Deviation #1). No animal required a salvage drench. Processed Groups 1 and 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0}C.
Day 21	12 SEP 13	Collected blood samples from animals in Groups 1, 2 and 3 for haemoglobin analysis, and faecal samples from Groups 1, 2 and 3 for FECs and group larval differentiation (see Deviation #1). No animal required a salvage drench. Processed Groups 1 and 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0}C. **Group 1 was vaccinated with IVP (V1) and Group 2 received IVP (V2).**
Day 28	19 SEP 13	Start of lambing.
Day 70	31 OCT 13	Marking: Collected blood samples from animals in Groups 1, 2 and 3 for haemoglobin analysis, and faecal samples from Groups 1, 2 and 3 for FECs and group larval differentiation. Several animals required a salvage drench (see Table 3 below). Processed Groups 1 and 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0}C. **Group 1 was vaccinated with IVP (V3) and Group 2 received IVP (V3)**. See **Amendment #1** and **NTF #4**.
Day 84	14 NOV 13	Collected blood samples from animals in Groups 1, 2 and 3 for haemoglobin analysis, and faecal samples from Groups 1, 2 and 3 for FECs and group larval differentiation. No animal required a salvage drench. Processed Groups 1 and 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0}C.
Day 98	28 NOV 13	Collected blood samples from animals in Groups 1, 2 and 3 for haemoglobin analysis, and faecal samples from Groups 1, 2 and 3 for FECs and group larval differentiation. No animal required a salvage drench. Processed Groups 1 and 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0}C.
Day 112	12 DEC 13	Weighed all sheep. Collected blood samples from animals in Groups 1, 2 and 3 for haemoglobin analysis, and faecal samples from Groups 1, 2 and 3 for FECs and group larval differentiation. No animal required a salvage drench. Processed Groups 1, 2 and 3 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0}C. **Group 1 was vaccinated with IVP (V3 + V 'spare' 19) and Group 2 received IVP (V4).**

Study Day*	Date	Event
Day 123	23 DEC 13	Collected blood samples from animals in Groups 1, 2 and 3 for haemoglobin analysis, and faecal samples from Groups 1, 2 and 3 for FECs and group larval differentiation. No animal required a salvage drench. Processed Groups 1 and 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0}C.
Day 140	09 JAN 14	Collected blood samples from animals in Groups 1, 2 and 3 for haemoglobin analysis, and faecal samples from Groups 1, 2 and 3 for FECs and group larval differentiation. Several animals required a salvage drench (see Table 3 below). Processed Groups 1 and 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0}C.
Day 154	23 JAN 14	Collected blood samples from animals in Groups 1, 2 and 3 for haemoglobin analysis, and faecal samples from Groups 1, 2 and 3 for FECs and group larval differentiation. Several animals required a salvage drench (see Table 3 below). Processed Groups 1 and 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0}C. **Group 1 was vaccinated with IVP (V4) and Group 2 received IVP (V5).**
Day 165	03 FEB 14	Weaning: Lambs were weaned off the ewes. The grazier “wet and dry” tested the ewes before moved them into new paddocks (see NTF #2 and NTF #3).
Day 167	05 FEB 14	Dispatch Plasma samples to Moredun. All Replica 1 plasma samples from Day 0 to Day 154.
Day 168	06 FEB 14	Collected blood samples from animals in Groups 1, 2 and 3 for haemoglobin analysis, and faecal samples from Groups 1, 2 and 3 for FECs and group larval differentiation. No animal required a salvage drench. Processed Groups 1 and 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0}C.
Day 182	20 FEB 14	Collected blood samples from animals in Groups 1, 2 and 3 for haemoglobin analysis, and faecal samples from Groups 1, 2 and 3 for FECs and group larval differentiation. No animal required a salvage drench. Processed Groups 1 and 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0}C.

Study Day*	Date	Event
Day 196	06 MAR 14	Collected blood samples from animals in Groups 1, 2 and 3 for haemoglobin analysis, and faecal samples from Groups 1, 2 and 3 for FECs and group larval differentiation. No animal required a salvage drench. Processed Groups 1 and 2 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{0}C. **Group 1 was vaccinated with IVP (V5) and Group 2 received IVP (V6).**
Day 210	20 MAR 14	Weighed all sheep. Collected blood samples from animals in Groups 1, 2 and 3 for haemoglobin analysis, and faecal samples from Groups 1, 2 and 3 for FECs and group larval differentiation. Processed Groups 1, 2 and 3 blood samples and harvested plasma. Plasma stored frozen in 2 replicates at approximately -20^{o}C. All animals were treated with COLLEAGUE to drench out animals.
	15 APR 14	Dispatch Plasma samples to Moredun. All remaining Replica 1 plasma samples.

**Note: In the protocol, amendments, deviations and raw data all activities are timed relative to lambing (Day 0 was 19 SEP 13) but in this report they are timed relative to the day of first vaccination (Day 0 is 22 AUG 13). This is a more accurate way of portraying the kinetics of the trial data because lambing was spread out over a few weeks (see NTF #6).*

10. TEST SYSTEM

Species: Ovine

Breed: Merino

Weight: 44.0–63.5 kg (Day 0) (see Deviation #3)

Sex: Maiden Merino ewes

Age: 24 months

Method of ID: Individually numbered eartags, coloured group eartags.

Number: 67

Source: Commercial sheep farm

Health & special requirements: Healthy animals. Not within existing WHP and ESI for animal health products used.

Not treated with a long-acting anthelmintic in the previous 2 months.

11. ANIMAL MANAGEMENT

a. Animal Welfare: Study animals were managed similarly and with due regard for their welfare. Animals were observed approximately twice weekly for health problems according to

AEC requirements. Animals were handled in compliance with UNE AEC no. 13-107 approved 01 AUG 13, and any applicable local regulations.

b. Concurrent Medications /Salvage Drenches: The grazier administered several concurrent medications over the duration of the trial as a part of normal husbandry and management practices.

- 09 SEP 13 – Glenvac 6-in-1 Injection (Batch: 06703, Expiry: NOV 15).
- 31 OCT 13 – 1 mL Cobalife Vitamin B12 + Se. (Batch: V10754/1, Expiry: JAN 17).
- 31 OCT 13 – 1 mL Glanvac 6-in-1 Injection (Batch: 09403, Expiry AUG 15)
- 22 JAN 14 – Animal #44 (Group 2) was treated for mastitis.

Salvage drenches that were administered on the basis of low haemoglobin content or high scour worm burden were recorded in the raw data (see NTF #1). A summary is provided below in Table 3.

Table 3: Salvage Drench Summary

Day	Animal ID	Group	Drench	Volume* (mL)	Salvage Drench
70	110	3	ZOLVIX	6.0	Low blood h/g
70	127	3	ZOLVIX	6.0	Low blood h/g
70	128	3	ZOLVIX	6.0	Low blood h/g
70	142	3	ZOLVIX	6.0	Low blood h/g
140	15	3	ZOLVIX	6.0	Low blood h/g
140	113	2	ZOLVIX	6.0	Low blood h/g
140	137	3	ZOLVIX	6.0	Low blood h/g
154	13	2	ZOLVIX	5.0	Low blood h/g
154	29	2	ZOLVIX	5.0	Low blood h/g
154	56	1	ZOLVIX	5.0	Low blood h/g
154	62	2	ZOLVIX	5.0	Low blood h/g
154	72	1	ZOLVIX	5.0	Low blood h/g
154	80	1	ZOLVIX	5.0	Low blood h/g
154	110	3	ZOLVIX	5.0	Low blood h/g
154	117	2	ZOLVIX	5.0	Low blood h/g
154	121	3	ZOLVIX	5.0	Low blood h/g
154	125	3	ZOLVIX	5.0	Low blood h/g
154	128	3	ZOLVIX	5.0	Low blood h/g
154	129	2	ZOLVIX	5.0	Low blood h/g
154	132	2	ZOLVIX	5.0	Low blood h/g
154	133	2	ZOLVIX	5.0	Low blood h/g
154	135	3	ZOLVIX	5.0	Low blood h/g
154	138	3	ZOLVIX	5.0	Low blood h/g
154	139	2	ZOLVIX	5.0	Low blood h/g

154	140	3	ZOLVIX	5.0	Low blood h/g
154	144	3	ZOLVIX	5.0	Low blood h/g
154	145	3	ZOLVIX	5.0	Low blood h/g

**Note: all animals were drenched to the highest weight in the group based upon Day 0 (22 AUG 13) or Day 112 (12 DEC 13) weights recorded. h/g = haemoglobin*

All animals in Groups 1, 2 and 3 were treated with an effective anthelmintic (ZOLVIX) on Day 0.

All animals in Groups 1, 2 and 3 were treated with an effective anthelmintic (COLLEAGUE) on Day 210.

c. Health Management: Study animals were clinically observed at each sampling time-point, no abnormalities were detected during the study. A single animal #62 (Group 2) was found dead in the paddock on 19 February 14 (Day 181). The ewe had been dead for approximately 48 hours and therefore an autopsy was not conducted. The death was not related to any treatments given during the trial.

d. Housing: Routine management practices were followed. All trial animals were maintained as a single group in one paddock (see NTF #3), with *ad-lib* access to a mixture of native and improved pastures and water from a dam.

e. Experimental diets: Not Applicable.

f. 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 obtained by the farmer and data from the nearest Bureau of Meteorology weather station for the study period are included in the raw data (see Appendix 5).

d. Sample Storage, Transfer & Disposal: Sample storage, transfer and disposal were recorded. Replicate 1 blood plasma samples were dispatched on dry-ice to Moredun Institute for analysis via World Courier on 05 February and 15 April 2014 with accompanying datalogger. Replicate 2 blood 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.

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 from each animal 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 & day, sample type, replicate. Frozen plasma samples were forwarded to Moredun Institute laboratories for haematology and biochemistry analysis on 05 Feb 14 and 15 Apr 14. Key haematological and biochemical parameters were compared to determine treatment effects, if any, and 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. Faecal egg counts and larval differentiation were performed (see Deviation #1). Faecal egg counts and larval differentiation were compared to determine treatment effects, if any, and are detailed in the results section of the Study Report.

14. STATISTICAL ANALYSIS

One-Way Analysis of Variance, its equivalent non-parametric test and additional statistical analysis may be performed as appropriate by the Sponsor's professional statisticians. See 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:

Amendment #1: The 'Marking' Day 56 (16^{th} October 2013) was changed to Day 70 (31^{st} October 2013). Some of the lambs were too young and risked being injured during the muster. This delay allowed us to work with the farmer; Ewes could be treated, bled and vaccinated (V3) on the same day, as would normally occur on a commercial sheep property (see Note to File #6). This amendment had no effect on the outcome of the trial.

b. Deviations:

Deviation #1: On Day 0 (22 August 2013), Day 14 (05 September 2013) and Day 21 (12 September 2013), the FEC samples were pooled into a single culture, instead of being cultured by groups, because of a misunderstanding between the Study Investigator and the Diagnostic lab staff. Since, every sample has been cultured by Group. This deviation had no effect on the outcome of the trial.

Deviation #2: The Sponsor did not provide an MSDS for the IVP 'BarberVax'. It wasn't deemed essential for pilot batches of the vaccine. This deviation had no effect on the outcome of the trial.

Deviation #3: In Section 10 Test system of the protocol it states that animals were to be between 30 – 55 kg. The weights of the animals used in the trial were outside those specifications outlined in the study protocol. All animals were in really good condition due to the season, and ewes were also in lamb. The ewes in Group 1 were animals used in another trial the previous year. Therefore these animals had to be used regardless of their weight. The other ewes that made up Groups 2 & 3 were the 'sisters' of the Group 1 ewes, as they are the same age and ran together as a mob. As a consequence, all animals were of the same age and were all 'maiden' ewes which were the requirement for the trial. This deviation had no effect on the outcome of the trial.

c. Notes to File:

Note to file #1: If the non *Haemonchus* egg count of an individual sheep, (calculated from the total egg count and the coproculture data) exceeded 1,500 epg that sheep was drenched at the next sampling date.

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.

Note to file #2: At the sponsor's request, all animals in the trial were 'wet' or 'Dry'. 'Wet' ewes were lactating as they were rearing a lamb. 'Dry' ewes (#62, #78, #128 and #144) did not raise a lamb for one reason or another.

Note to file #3: In this study, lambs were weaned from the ewes on Monday 3rd February 2014, later than usual due to the drought and little feed or water in the paddocks. Ewes were moved into new 'clean' paddocks due to feed restrictions.

Note to file #4: V2 was spilt travelling to Dundee. Consequently, both groups 1 and 2 received V3.

Note to file #5: Animals #32 and #39 (both Group 1) raised twin lambs.

Note to file #6:

a) In the protocol, amendments, deviations and raw data all activities are timed relative to lambing (Day 0 was 19 SEP 13) but in this report they are timed relative to the day of first vaccination (Day 0 is 22 AUG 13). This is a more accurate way of portraying the kinetics of the trial data because lambing was spread out over a few weeks.

b) Clarification of terms in Amendment #1: the day 'Marking' referred to all the activities that were to occur of that day (regardless of date) as out lined in the study protocol. I.e. Group 1 received V2, Group 2 received V3 and all animals were also bled, haemoglobin analysis conducted, FEC'ed and cultured.

d. Change of Study Personnel: Not applicable

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 Reports and Statistical Reports 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: One ewe from the First Vaccinated group (animal #62) died (19 FEB 14). Its data was excluded from the analysis.

b. Lambing success and number of ewes used in the study: There were 67 ewes at the start of the trial but four, two each in the First Vaccinated and Control groups, did not raise a lamb, either because they were not pregnant, or their lamb was born dead or died soon after birth. These 4 sheep remained with the rest of the flock throughout the trial but their data was excluded from the analysis.

Of the 63 surviving ewes which raised a lamb (all singles, except for 2 sets of twins in the Previously Vaccinated group), 17 had been previously vaccinated, 23 were vaccinated for the first time and 23 were Controls.

c. Types of comparison made: It was of particular interest to know how well the vaccine performed during the "periparturient" phase of the trial because worm eggs shed during lactation are an important source of infection for the next generation of lambs and hence the general epidemiology of Haemonchosis in a flock. Therefore the degree of protection attained from lambing to weaning is presented in addition to that calculated for the whole duration of the trial.

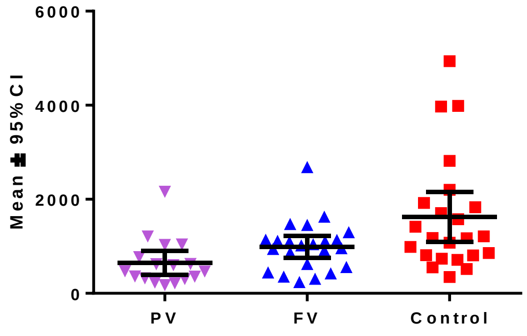
d. Rainfall and pasture infectivity: The 2013-2014 summer was exceptionally dry in New England and the trial site was no exception (Table A6, Fig 6). However, as can be seen from the egg counts of those sheep which received a precautionary drench, larvae were still being picked up in February despite little rain that month (Table A1, Fig 3).

e. *Haemonchus* Egg Counts:

iii) From lambing to weaning

During lactation the overall averaged egg counts were reduced by 58.6 % in the Previously Vaccinated sheep compared to the Controls (Fig 1), a statistically significant difference but differences between the First Vaccinated and Control or Previously Vaccinated groups were not statistically significant.

Fig 1. Individual ewe counts averaged over lactation



iv) Over the whole trial

Overall averaged *Haemonchus* egg counts were significantly reduced in both vaccinated groups of sheep compared to the Controls (Fig 2) but no significant difference was detected between the groups of vaccinates.

A single "non-responder" (defined as a vaccinated animal with a mean egg count greater than the 95% lower confidence limit of the control group) was identified in the Previously Vaccinated group.

Fig 2 Individual ewe egg counts averaged over the trial

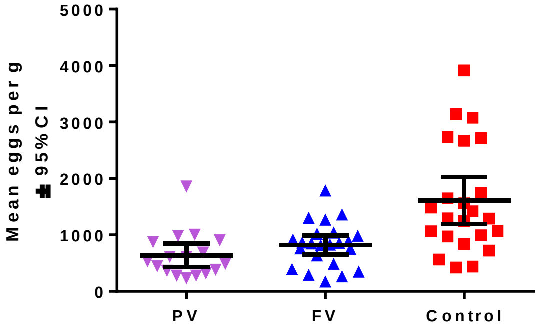


Table 4: Percent protection (eggs) on each sampling day

	Days	PV mean	%P	FV mean	%P	Control mean	P value after ANOVA: PV vs Cont	FV vs Cont	PV vs FV
V1	0	**240**	71.3	**435**	47.9	**834**	n/s	n/s	n/s
	14								
V2	21								
V3, marking	70	**395**	47.0	**1033**	-38.6	**745**	n/s	n/s	**
	84	**538**	57.2	**998**	20.7	**1259**	***	n/s	*
	98	**246**	44.3	**558**	-26.1	**442**	n/s	n/s	n/s
V4	112	**175**	48.5	**258**	24.1	**340**	n/s	n/s	n/s
	123	**417**	64.6	**726**	38.5	**1179**	*	n/s	n/s
	140	**1144**	66.1	**1972**	41.5	**3370**	****	*	n/s
V5	154	**1606**	57.1	**1400**	62.6	**3746**	n/s	*	n/s
weaned d165	168	**547**	53.0	**469**	59.7	**1163**	n/s	n/s	n/s
	182	**572**	65.0	**872**	46.6	**1634**	*	n/s	n/s
V6	196	**534**	34.0	**390**	51.7	**808**	n/s	**	n/s
	210	**751**	65.1	**523**	75.7	**2151**	**	****	*

n/s: non-significant; * P<0.05; ** P<0.02; *** P<0.01; **** P<0.001;

f. Kinetics of and relationships between the parameters studied over the course of the trial: Antibody titres in the unvaccinated control ewes remained at background levels close to zero throughout the trial (Table A4, Fig 3). In contrast, group mean titres in the Previously Vaccinated group were around 7,000 from the beginning of the trial where they remained until their second vaccination at marking time. After that they rose to about 10,000, staying at this level until their final boost, when they increased once again. Meanwhile, titres in the First Vaccinated ewes responded to each vaccine boost with a temporary spike in titre observed at the next sampling. Mean titres in the Previously Vaccinated ewes always exceeded those of the First Vaccinates by at least two-fold (Fig 3).

Total and *Haemonchus* specific group mean faecal egg counts were quite similar and followed very similar patterns in all groups, reflecting the fact that *Haemonchus* was usually the dominant gastrointestinal nematode genus infecting the ewes (Fig 3, Table A2).

Mean Control egg counts, which were about 1000 at the start of the trial, dropped to zero after the anthelmintic given then, before recovering to initial levels by marking time. Thereafter mean Control egg counts dipped during dry December before peaking to some 3,700 during January and then fluctuating in the 1-2000 range for the remainder of the trial. Mean *Haemonchus* egg counts in the Previously Vaccinated ewes were always lower than those of the Controls and often this difference was statistically significant (Table 4), but those of the First Vaccinated group tended to be intermediate, although they were significantly protected on three occasions (Table 4).

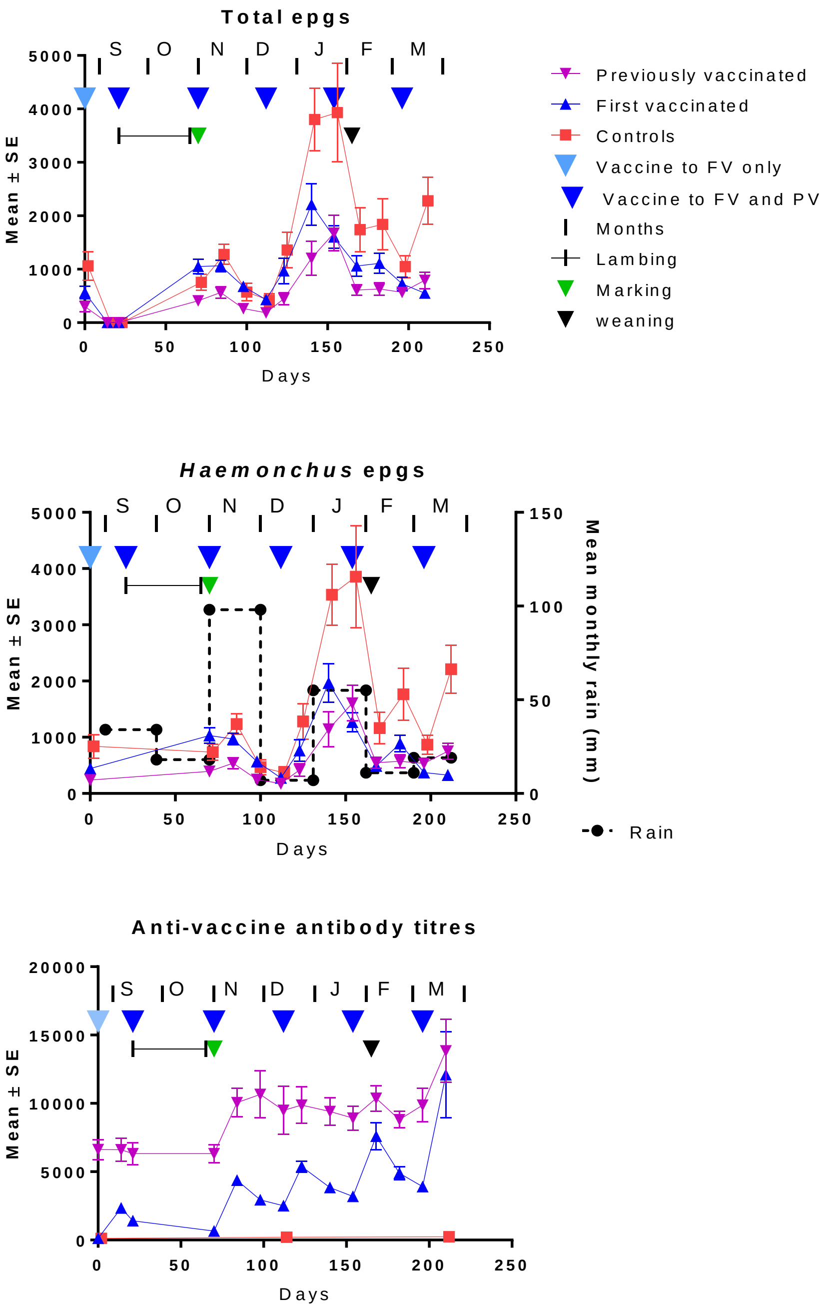


Fig 3. Kinetics of interventions, group mean total egg counts, *Haemonchus* specific egg counts and the anti-vaccine antibody response

The kinetics and group differences in *Haemonchus* specific egg counts were inversely reflected in the degree of anaemia and precautionary drenching (Table 3, Fig 4). Thus, peak egg counts in January coincided with the lowest blood haemoglobin concentrations (Table A3) and when most precautionary drenching was required (Fig 4). Although there were few occasions when there were significant differences between the groups in terms of blood haemoglobin, the vaccinates needed significantly fewer precautionary drenches than the controls, with the Previously Vaccinated group requiring less treatment than those vaccinated for the first time (Table 5).

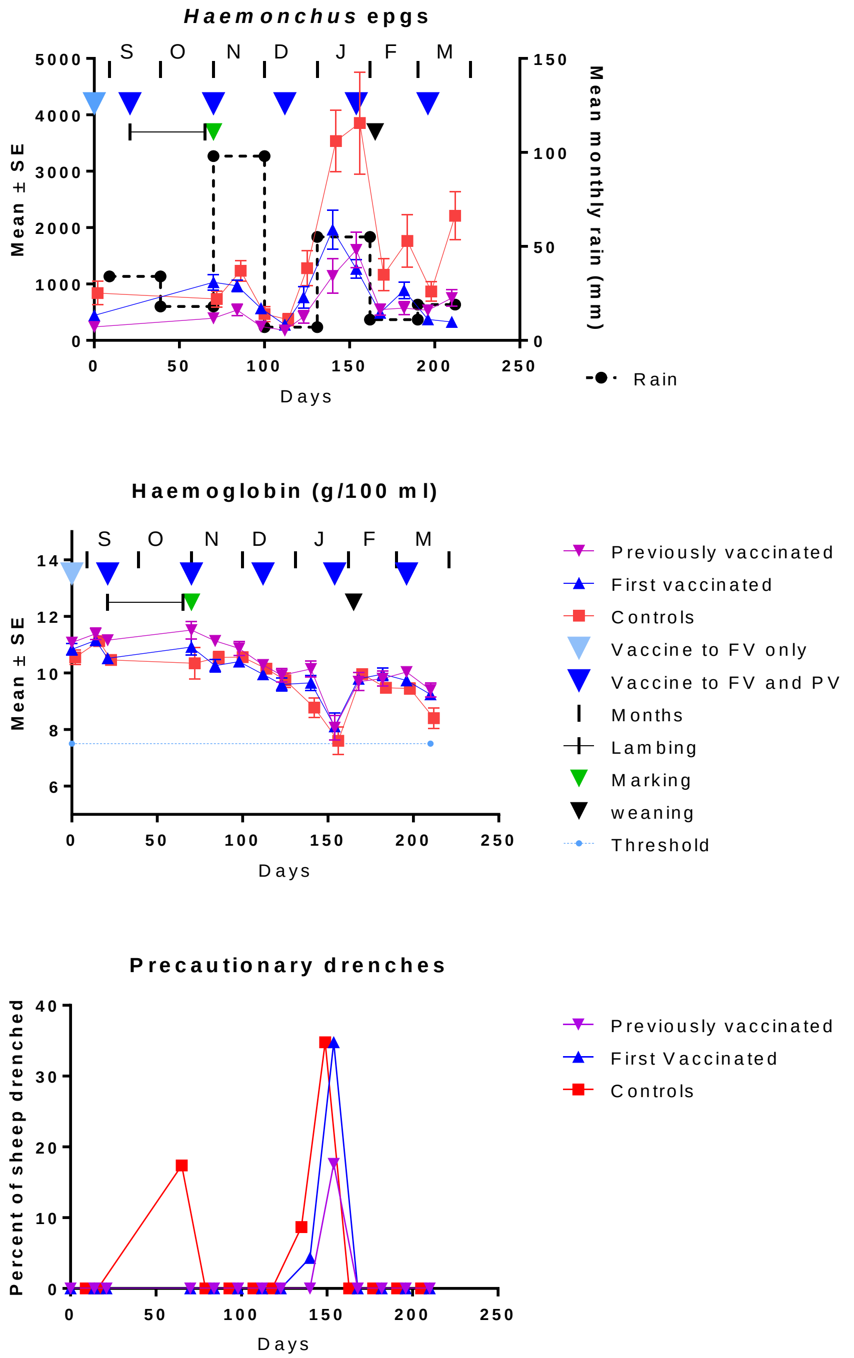


Fig 4. Kinetics of the *Haemonchus* specific egg counts in relation to blood haemoglobin concentrations and to precautionary drenching.

Table 5: Precautionary drenches

	Number given			**Percent of group treated**			**P by Fishers exact test**		
sheep/ group	17	23	23						
Days	**PV**	**FV**	**C**	**PV**	**FV**	**C**	**PV vs C**	**FV vs C**	**PV vs FV**
0	0	0	0	0	0	0			
14	0	0	0	0	0	0			
21	0	0	0	0	0	0			
70	0	0	4	0	0	17.4	****	****	n/s
84	0	0	0	0	0	0			
98	0	0	0	0	0	0			
112	0	0	0	0	0	0			
123	0	0	0	0	0	0			
140	0	1	2	0	4.3	8.7	**	n/s	n/s
154	3	8	9	17.6	34.8	39.1	**	n/s	**
168	0	0	0	0	0	0			
182	0	0	0	0	0	0			
196	0	0	0	0	0	0			
210	0	0	0	0	0	0			
Total	**3**	**9**	**15**	**17.6**	**39.1**	**65.2**	****	**	**

n/s: non-significant; * P<0.05; ** P<0.02; *** P<0.01; **** P<0.001;

g. Ewe bodyweights: The Previously Vaccinated ewes were significantly heavier than the First Vaccinated sheep at the start of the trial and this difference was maintained at the half way point, though had disappeared by the end of the trial (Table A5, Fig 5). More striking was the overall decline in bodyweight in all three groups (Fig 5).

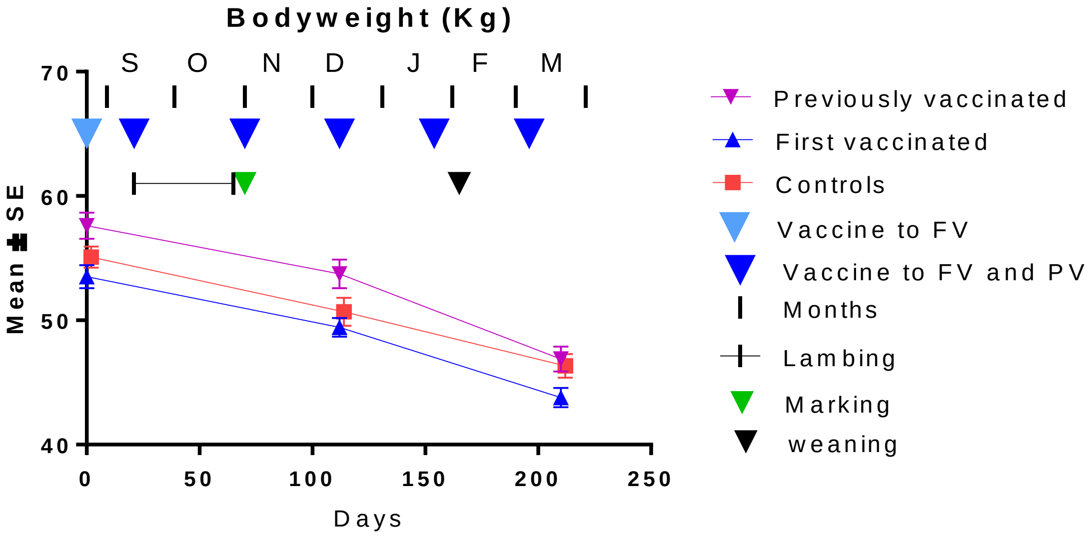


Fig. 5 Ewe bodyweights over the course of the trial.

18. Concluding Remarks

It was concluded that Barbervax was capable of suppressing *Haemonchus* egg counts in periparturient and lactating ewes, though the effect was stronger in sheep which had received a course of the vaccine in an earlier season. This was an important finding because the eggs shed by lactating ewes are an important source of contamination for their lambs and hence the epidemiology of *Haemonchus*. In addition the vaccine provided a distinct benefit to the ewes themselves, reducing the proportion which required anthelmintic support to prevent potentially fatal anaemia.

APPENDIX 3

TABULATED AND RAW DATA

Table A1: Total strongyle egg counts (epg)

	Event		**V1**		**V2**	**V3**				**V4**		**V5**			**V6**	
	Date		22-Aug	05-Sep	12-Sep	31-Oct	14-Nov	28-Nov	12-Dec	23-Dec	09-Jan	23-Jan	06-Feb	20-Feb	06-Mar	20-Mar
		Days	**0**	**21**	**28**	**70**	**84**	**98**	**112**	**123**	**140**	**154**	**168**	**182**	**196**	**210**
Group	**Ewe#**	**lamb**														
Prev V	1	Single	640	0	0	400	440	240	80	480	1040	320	640	1040	360	560
Prev V	12	Single	800	0	0	280	280	320	0	280	1920	320	640	40	800	240
Prev V	23	Single	0	0	0	320	320	120	120	200	240	280	280	360	440	560
Prev V	25	Single	400	0	0	920	320	160	160	360	1240	1360	1600	1440	1720	760
Prev V	32	**Twin**	40	0	0	1120	1600	160	80	120	880	1680	680	40	520	160
Prev V	33	Single	240	0	0	200	440	360	80	160	600	2560	240	360	120	480
Prev V	39	**Twin**	80	0	0	200	280	80	40	160	120	1480	680	520	600	280
Prev V	44	Single	200	0	0	120	240	120	200	440	640	2800	400	600	1120	1200
Prev V	48	Single	120	0	0	240	880	400	360	480	680	4480	720	880	600	680
Prev V	50	Single	520	0	0	280	280	120	80	200	440	1240	160	560	440	440

Prev V	54	Single	0	0	0	240	240	0	80	0	120	600	400	440	280	320
Prev V	56	Single	240	0	0	1320	1280	640	840	1840	5480	4360	80	600	640	2360
Prev V	60	Single	0	0	0	0	200	120	120	320	1120	720	720	1720	400	680
Prev V	66	Single	120	0	0	240	840	400	360	120	840	640	1040	1320	200	1040
Prev V	68	Single	120	NS	0	280	40	200	80	360	320	400	320	600	640	440
Prev V	72	Single	1640	0	0	440	920	400	40	1280	1800	2720	0	200	280	2200
Prev V	80	Single	0	0	0	400	1000	640	440	800	3000	2560	0	40	520	1080
First V	62	**None**	160	0	0	1480	1800	760	160	680	4400	5520	0	dead	dead	dead
First V	78	**None**	640	0	0	680	NS	240	0	40	200	1720	320	640	1600	8560
First V	7	Single	560	0	NS	2680	920	840	560	1600	4520	440	1320	3400	800	760
First V	10	Single	120	0	0	2280	1040	600	320	600	1960	760	1560	2440	640	600
First V	13	Single	40	0	0	1760	1600	600	360	1120	2640	720	0	520	640	680
First V	18	Single	0	0	0	1040	1040	520	240	280	640	680	320	1000	560	360
First V	29	Single	400	0	0	960	1560	800	600	2240	640	1720	0	480	0	40
First V	37	Single	80	0	0	520	280	280	80	280	560	480	400	480	840	80
	Event		**V1**		**V2**	**V3**				**V4**		**V5**			**V6**	

Study no. MIHO2920

		Date	22-Aug	05-Sep	12-Sep	31-Oct	14-Nov	28-Nov	12-Dec	23-Dec	09-Jan	23-Jan	06-Feb	20-Feb	06-Mar	20-Mar
		Days	**0**	**21**	**28**	**70**	**84**	**98**	**112**	**123**	**140**	**154**	**168**	**182**	**196**	**210**
Group	**Ewe#**	**lamb**														
First V	71	Single	840	0	0	640	1040	800	800	640	2840	2360	920	760	800	520
First V	113	Single	640	0	0	760	1080	1480	1160	5480	9040	0	80	600	760	1480
First V	115	Single	1240	0	40	1200	2360	1760	280	560	3320	2440	2200	2320	1760	1520
First V	117	Single	120	0	0	1560	1040	120	120	120	2640	3640	40	160	120	0
First V	118	Single	760	0	NS	840	1080	440	360	1200	2200	1640	1480	1600	1320	120
First V	119	Single	400	0	0	1840	1040	680	560	600	2320	2000	920	1160	1840	1200
First V	122	Single	280	0	0	480	920	520	0	240	2080	2840	2160	880	80	1040
First V	123	Single	120	0	0	560	1080	520	120	560	2920	2800	2520	2280	1440	680
First V	124	Single	1360	0	0	360	640	480	480	800	960	1560	560	1680	800	1080
First V	126	Single	880	0	0	440	440	560	360	40	720	960	560	640	840	280
First V	129	Single	1240	0	0	1480	1920	1000	1440	1160	1160	1280	0	360	520	320
First V	131	Single	120	0	0	200	360	120	40	560	960	640	40	320	160	200
First V	132	Single	2280	0	0	920	1280	920	520	1720	3360	1960	0	640	120	920
First V	133	Single	200	0	0	160	40	80	80	40	680	920	0	0	40	80

First V	134	Single	120	0	0	1120	1440	1240	NS	280	760	1400	600	1720	2160	120
First V	139	Single	1160	0	0	760	800	80	400	360	760	440	40	160	80	360
First V	141	Single	0	0	0	1640	1520	1160	640	1760	3120	3600	1360	1920	440	280
Control	128	**None**	1920	0	0	0	0	0	0	0	160	440	0	0	120	320
Control	144	**None**	0	0	0	1760	3000	280	40	40	3000	4880	0	280	160	2800
Control	4	Single	40	0	0	720	920	440	160	600	2680	1800	4160	5720	2520	NS
Control	15	Single	3640	0	0	1440	2480	1760	2080	5520	12440	2800	160	560	960	6320
Control	26	Single	240	0	0	1360	2920	2560	640	1520	4800	7160	4480	2800	1320	3280
Control	35	Single	0	0	0	1000	680	80	0	120	2160	40	280	280	280	120
Control	64	Single	200	0	0	120	680	280	120	280	1840	2120	2400	2240	1880	3200
Control	110	Single	0	0	80	1920	0	0	640	3160	4880	320	40	120	480	3400
Control	111	Single	40	0	0	240	840	400	280	120	1080	880	720	1520	400	2120
Control	112	Single	600	0	0	680	1520	160	0	720	1760	1080	800	1000	400	1800
Control	114	Single	3160	0	NS	160	640	200	160	200	2720	1200	480	240	320	400
Control	116	Single	3600	0	NS	40	NS	0	40	320	3200	3800	1040	920	760	440
	Event		**V1**		**V2**	**V3**				**V4**		**V5**			**V6**	

Study no. MIHO2920

	Date		22-Aug	05-Sep	12-Sep	31-Oct	14-Nov	28-Nov	12-Dec	23-Dec	09-Jan	23-Jan	06-Feb	20-Feb	06-Mar	20-Mar
		Days	**0**	**21**	**28**	**70**	**84**	**98**	**112**	**123**	**140**	**154**	**168**	**182**	**196**	**210**
Group	**Ewe#**	**lamb**														
Control	120	Single	40	0	0	400	1080	120	NS	120	1800	3800	3960	7240	3160	7480
Control	121	Single	2280	0	0	280	1080	320	160	960	2680	3440	0	200	200	4200
Control	125	Single	3120	40	0	2520	1680	1800	2160	5440	7320	15480	0	480	280	NS
Control	127	Single	1720	0	0	NS	0	0	0	80	280	1440	800	1040	400	320
Control	130	Single	40	0	0	920	2320	880	240	880	2360	440	800	1160	1480	520
Control	135	Single	200	0	0	640	680	320	0	120	3120	6600	0	1240	2000	2560
Control	136	Single	0	0	0	160	NS	40	160	520	1400	2800	2880	2640	880	560
Control	137	Single	1440	0	0	240	480	320	360	1840	8600	0	0	1240	1040	3520
Control	138	Single	1640	0	0	200	800	640	480	3000	3680	1800	0	920	240	1960
Control	140	Single	1040	0	0	2000	2760	2440	520	1360	7000	13320	0	440	480	2080
Control	142	Single	1200	0	0	280	0	0	280	2560	1840	520	600	1440	NS	2720
Control	143	Single	200	NS	NS	440	640	440	680	1640	4840	7520	4280	8720	3480	320
Control	145	Single	40	0	0	920	760	0	80	240	4960	7040	0	120	120	560

Note: Animals were drenched due to low haemoglobin blood concentration (<6.5g/100mL)

Table A2: Results of coprocultures – percent of each species of nematode larvae

Day	Group	Treatment	Haem	Trich	Ost	Coop	Oes	TOTAL
	1	Previous IVP						
-28	2	IVP	79%	11%	10%			100%
	3	Control						
	1	Previous IVP						
-14	2	IVP			100%			
	3	Control						
	1	Previous IVP						
-7	2	IVP		100%				
	3	Control						
	1	Previous IVP	96%		4%			100%
42	2	IVP	98%	1%	1%			100%
	3	Control	97%	1%	2%			100%
56	1	Previous IVP	82%	10%	7%	1%		100%

Day	Group	Treatment	Haem	Trich	Ost	Coop	Oes	TOTAL
	2	IVP	91%	3%	6%			100%
	3	Control	97%		3%			100%
70	1	Previous IVP	50%	33%	13%	4%		100%
	2	IVP	84%	5%	8%	1%	2%	100%
	3	Control	82%	9%	4%		5%	100%
82	1	Previous IVP	26%	25%	42%		7%	100%
	2	IVP	64%	3%	27%	1%	5%	100%
	3	Control	88%	7%	5%			100%
95	1	Previous IVP	53%	38%	9%			100%
	2	IVP	79%	12%	1%	7%	1%	100%
	3	Control	94%	6%				100%
112	1	Previous IVP	75%	17%	8%			100%
	2	IVP	89%	3%	6%		2%	100%
	3	Control	93%	5%	2%			100%
126	1	Previous IVP	72%	28%				100%

Day	Group	Treatment	Haem	Trich	Ost	Coop	Oes	TOTAL
	2	IVP	79%	15%	5%	1%		100%
	3	Control	98%	2%				100%
140	1	Previous IVP	14%	39%	43%	4%		100%
	2	IVP	46%	18%	33%		3%	100%
	3	Control	71%	18%	11%			100%
154	1	Previous IVP	37%	32%	19%	2%	10%	100%
	2	IVP	80%	15%	3%		2%	100%
	3	Control	96%	3%			1%	100%
168	1	Previous IVP	34%	48%	13%		5%	100%
	2	IVP	51%	18%	30%		1%	100%
	3	Control	83%	16%	1%			100%
182	1	Previous IVP	65%	18%	13%		4%	100%
	2	IVP	59%	36%	4%		1%	100%
	3	Control	97%	3%				100%

Table A3: Blood Haemoglobin concentrations (g/100ml)

Days

[yellow] = Hb<6.5 g/100ml

Group	EWE#	0	21	28	70	84	98	112	123	140	154	168	182	196	210
Prev V	1	10.2	11.9	11.2	12.5	10.8	10.7	9.3	9.9	8.9	8.6	8.8	9.2	9.3	8.5
Prev V	12	10.1	11.4	12.1	8.6	10.4	9.6	10.6	10.8	9.6	9.0	10.2	7.7	10.9	10.3
Prev V	23	10.0	10.6	10.5	10.9	9.9	9.3	8.9	9.1	9.8	9.8	8.2	9.5	9.6	8.0
Prev V	25	11.2	12.0	10.9	11.5	11.1	11.1	10.0	10.1	9.2	9.0	9.2	8.2	9.4	9.0
Prev V	32	11.4	12.3	12.3	12.5	11.3	10.7	10.6	11.6	11.6	9.6	11.9	10.9	10.2	11.2
Prev V	33	12.3	10.7	10.0	12.1	11.6	11.4	9.8	9.2	9.9	10.4	9.6	10.1	10.5	9.8
Prev V	39	11.3	11.6	11.4	11.7	11.2	10.5	9.9	9.4	10.3	5.8	10.3	9.7	10.3	8.9
Prev V	44	11.4	10.0	11.5	12.1	12.1	12.1	11.2	11.3	11.6	7.8	8.1	9.3	10.0	8.9
Prev V	48	12.3	12.5	11.9	9.3	10.2	11.5	10.7	11.2	12.3	10.1	11.8	10.6	11.3	10.9
Prev V	50	10.5	11.7	11.8	12.4	11.1	10.7	11.1	9.8	10.5	7.2	10.2	9.9	9.5	10.9
Prev V	54	10.7	11.3	9.9	13.3	11.9	11.5	11.0	9.6	10.1	10.1	9.1	8.1	8.7	8.9
Prev V	56	10.8	11.2	11.0	10.1	10.3	8.8	9.1	7.6	7.8	7.4	9.9	10.8	10.3	7.4
Prev V	60	11.0	13.2	11.6	11.9	12.5	12.1	10.2	10.5	11.0	7.4	6.9	10.8	10.0	10.0
Prev V	66	11.2	10.9	11.6	11.8	11.8	11.3	11.3	8.9	10.4	6.0	9.6	10.3	9.1	8.7

Prev V	68	12.2	11.2	11.7	13.0	12.0	12.3	11.5	10.4	11.2	4.7	10.5	11.0	11.0	9.6
Prev V	72	10.1	10.2	9.8	10.6	9.9	10.1	10.0	9.3	8.9	8.8	9.1	9.1	9.4	9.8
Prev V	80	11.7	10.9	10.6	11.6	11.2	11.1	9.7	10.0	9.4	5.5	11.4	11.5	11.2	9.1
First V	7	10.8	11.8	10.7	9.9	7.3	10.1	10.1	9.6	8.6	7.2	9.2	8.9	9.5	9.5
First V	10	10.8	10.8	10.7	11.0	10.4	9.8	10.5	10.1	10.6	10.8	8.8	9.0	10.3	9.9
First V	13	12.4	11.0	11.0	11.8	11.1	9.5	10.0	9.4	8.9	10.0	10.0	10.1	10.9	9.3
First V	18	11.0	10.3	10.2	12.8	9.8	9.3	8.2	8.6	9.7	9.4	9.0	9.3	9.8	8.2
First V	29	10.4	10.9	10.4	10.2	10.2	9.6	10.5	8.9	8.9	10.6	10.7	10.3	10.4	9.7
First V	37	11.3	11.4	10.7	11.8	11.1	11.6	11.4	NS	11.1	12.7	10.1	10.1	10.1	9.3
First V	62	11.4	10.8	10.4	10.9	10.6	10.0	10.0	9.2	8.8	10.2	10.2			
First V	71	11.5	12.1	11.6	7.1	12.2	11.4	10.4	9.9	10.7	4.7	10.1	10.7	9.8	9.1
First V	78	11.5	12.1	7.7	10.6	8.7	11.0	11.3	13.0	13.1	8.5	9.8	12.0	8.5	9.0
First V	113	9.6	10.9	10.2	12.0	11.2	10.5	8.1	6.6	6.3	6.8	9.4	8.1	9.7	8.8
First V	115	10.3	11.0	11.0	10.2	9.6	9.9	9.7	9.3	8.9	3.8	10.1	10.1	10.1	9.7
First V	117	10.1	11.2	10.3	10.3	8.9	9.6	9.7	9.0	8.3	9.1	8.9	9.3	8.8	9.6
First V	118	11.1	11.4	11.6	12.3	11.3	10.4	9.6	9.2	8.4	10.7	10.0	10.2	9.5	9.5

Group	EWE#	0	21	28	70	84	98	112	123	140	154	168	182	196	210
First V	119	11.2	12.2	11.3	13.6	10.4	11.2	10.7	10.4	9.5	5.6	9.9	10.8	7.9	10.0
First V	122	11.1	11.5	11.0	12.4	11.6	10.9	10.1	9.5	8.1	7.8	9.0	9.8	9.9	10.5
First V	123	14.1	12.7	11.4	12.1	11.8	11.4	10.9	10.6	10.6	10.0	10.6	9.3	10.5	9.7
First V	124	10.7	10.3	12.4	11.0	9.5	10.7	9.3	9.2	9.5	9.9	9.7	11.0	9.4	8.3
First V	126	8.9	9.6	9.9	8.4	9.5	10.2	9.7	9.4	9.8	9.6	9.9	8.4	8.6	8.4
First V	129	10.1	10.6	9.0	8.9	9.3	9.9	9.7	8.1	9.3	6.4	9.9	11.0	10.4	7.2
First V	131	10.3	10.2	10.6	11.2	10.7	9.1	8.4	10.1	10.6	5.8	10.1	11.0	7.5	7.0
First V	132	9.9	11.4	9.8	11.6	11.5	11.2	9.9	9.8	9.3	8.6	10.0	7.4	11.1	10.5
First V	133	11.1	10.6	11.2	10.1	9.9	10.4	10.3	10.9	11.6	4.9	10.8	10.3	9.6	10.0
First V	134	10.6	11.9	10.1	11.1	10.4	10.9	9.7	9.3	10.5	4.2	10.9	10.7	10.4	9.1
First V	139	9.8	10.3	9.7	10.9	10.3	10.8	10.5	10.5	10.5	6.9	9.6	11.1	10.7	9.4
First V	141	10.9	11.9	10.2	10.8	9.3	10.8	10.1	9.6	9.8	8.7	8.2	10.2	10.4	10.1
Control	4	11.3	11.4	10.5	12.2	10.4	10.1	9.9	9.7	10.2	9.4	9.7	9.8	8.2	7.4
Control	15	8.9	11.0	10.9	12.8	11.3	10.4	9.2	7.8	4.3	9.6	10.6	9.2	9.5	7.8
Control	26	12.5	11.9	11.4	11.8	10.2	10.1	10.4	9.8	7.8	4.7	9.1	9.6	9.7	8.5
Control	35	10.8	12.1	11.1	11.2	12.0	11.1	11.2	10.5	9.6	9.9	10.0	9.8	9.5	10.3

Control	64	10.8	10.6	10.3	11.8	11.6	11.6	10.8	10.8	10.2	7.7	9.9	10.6	10.1	7.5
Control	110	13.1	13.1	9.2	5.0	9.7	9.7	9.4	7.5	6.9	4.7	9.3	8.0	8.3	8.0
Control	111	13.1	11.5	11.0	12.7	11.7	12.0	11.1	11.3	11.2	10.6	10.5	9.1	10.3	10.0
Control	112	10.9	11.6	11.7	11.7	10.2	9.2	9.8	9.5	8.7	7.4	10.0	8.6	9.1	9.6
Control	114	11.1	11.7	10.6	13.5	12.5	12.0	9.1	9.8	9.7	3.5	10.8	11.2	11.2	11.7
Control	116	10.1	10.9	NS	NS	NS	12.6	11.3	12.3	10.2	11.0	11.7	9.7	10.3	10.9
Control	120	10.3	10.9	10.4	12.0	10.9	10.6	10.3	10.0	8.2	8.0	8.5	9.6	7.6	4.1
Control	121	9.3	9.7	10.4	10.5	10.3	10.2	8.9	8.3	7.5	10.4	9.1	10.4	10.0	6.3
Control	125	11.4	12.2	11.9	7.6	10.4	10.8	9.8	9.3	7.3	7.8	8.8	8.2	9.3	8.7
Control	127	8.8	10.8	11.8	6.2	9.4	9.9	10.2	10.2	11.2	11.8	9.2	9.7	7.8	8.0
Control	128	8.1	8.9	9.4	3.8	8.8	9.8	10.1	10.0	10.5	3.7	10.5	10.8	9.6	7.9
Control	130	10.6	11.3	9.8	11.4	10.3	10.0	10.7	10.7	9.3	6.3	11.9	7.9	10.4	10.1
Control	135	10.5	11.1	10.8	12.2	12.5	11.7	11.3	10.4	8.9	8.9	11.7	8.9	10.2	8.6
Control	136	9.3	10.7	10.6	7.3	10.2	10.8	11.1	10.9	10.1	4.3	8.9	8.7	8.9	9.4
Control	137	9.3	10.6	10.6	12.6	10.7	10.0	8.6	7.3	6.2	7.5	10.1	10.1	8.6	5.4
Control	138	10.0	10.2	9.5	11.5	9.5	9.3	8.2	7.8	7.4	7.8	9.4	9.6	9.3	7.4

Group	EWE#	0	21	28	70	84	98	112	123	140	154	168	182	196	210
Control	140	9.8	10.3	11.0	10.2	9.2	9.0	9.1	9.1	6.9	9.4	10.1	10.5	8.2	6.4
Control	142	10.3	11.3	10.4	5.9	8.5	10.2	9.6	8.6	8.8	6.0	10.1	9.8	8.6	7.4
Control	143	10.9	11.5	9.8	10.3	10.8	10.8	10.8	10.3	9.0	8.8	10.4	7.6	10.3	11.2
Control	144	12.5	11.3	7.5	11.8	10.9	10.9	11.7	11.7	11.0	6.2	9.2	9.3	11.4	8.3
Control	145	10.1	11.7	10.5	12.4	11.3	11.3	11.2	10.0	8.3	4.7	9.6	10.3	10.0	9.2

Table A4: ELISA titres

Group	Ewe #	Days													
		0	**14**	**21**	**70**	**84**	**98**	**112**	**123**	**140**	**154**	**168**	**182**	**196**	**210**
Prev V	**1**	6328	6205	6263	6097	10545	9963	8352	7531	7022	7758	9539	8530	9981	16110
Prev V	**12**	5608	5487	5557	4683	9938	7679	7211	10675	10213	9013	7643	6554	7877	7221
Prev V	**23**	4206	3405	2954	3601	9645	7967	7172	12948	8070	8148	9903	7639	8087	12759
Prev V	**25**	4607	4451	3537	3907	7890	6841	6077	6931	6088	6375	7827	6235	6686	13718
Prev V	**32**	7662	7431	7298	5631	9140	8336	7597	8647	7895	7869	8399	7138	7810	10275
Prev V	**33**	8117	8876	8276	8750	17107	26164	15402	12994	14123	15586	12247	10377	13307	18075
Prev V	**39**	15012	16433	14462	14852	22904	26957	35468	28636	22062	20207	24021	17095	28000	49206
Prev V	**44**	11143	11471	11003	8416	13284	22482	14370	11268	15015	10435	10725	9235	12750	13925
Prev V	**48**	7133	7116	6764	6074	7811	7273	6885	8954	7013	7188	9737	8107	8398	10580
Prev V	**50**	7963	7895	9292	6572	9207	8559	8051	8800	7781	7449	7817	8312	8705	12320
Prev V	**54**	2259	1744	1382	3510	5976	5458	5152	5695	6867	6164	10189	7888	7220	9549
Prev V	**56**	4355	4110	3713	3986	5462	4857	4643	4306	5294	5218	6563	6441	6790	9257
Prev V	**60**	7763	7416	7428	7103	9537	9232	8181	3990	8826	8899	10595	8844	9550	10378
Prev V	**66**	5955	5751	5698	7743	8385	7705	7936	7387	9580	7833	11160	9706	8235	11238

Group	Ewe #	Days													
		0	**14**	**21**	**70**	**84**	**98**	**112**	**123**	**140**	**154**	**168**	**182**	**196**	**210**
Prev V	**68**	6662	6822	7129	6112	9060	8444	7514	11268	8857	9440	8220	8226	7251	10291
Prev V	**72**	3595	3164	2891	4964	7519	6512	5823	8954	7803	7433	11366	9781	8876	10720
Prev V	**80**	4215	4541	3897	5528	7662	6633	5637	8800	7400	6803	10476	9693	8261	9599
First V	**7**	0	25	1	613	5656	3492	2961	5695	4192	2986	7356	5052	3444	12051
First V	**10**	0	1899	494	139	4296	2010	1867	4306	3089	2074	4118	2673	1411	5477
First V	**13**	437	1885	992	2172	4278	2831	2592	3990	3007	2251	6344	5386	4056	10586
First V	**18**	19	258	10	56	4381	2160	2020	7387	6473	5308	9446	7692	5749	12767
First V	**29**	3	488	45	221	3528	2043	1443	827	356	652	3381	1669	1002	4728
First V	**37**	1	1841	695	131	5232	4095	3724		5794	5331	16539	8268	6545	14248
First V	**62**	6	568	54	151	2846	1512	928	3510	2344	1822	4722			
First V	**71**	138	2160	993	440	3430	2099	2363	7392	4416	3399	7220	4656	3464	6060
First V	**78**	108	2208	279	844	3061	1706	1367	4312	2631	2389	4032	2543	1812	3067
First V	**113**	195	2109	7355	193	5460	3708	3225	5378	2904	2527	5022	3942	2953	6945
First V	**115**	370	3121	567	1310	3590	2168	2603	2885	1887	2013	5174	4414	3734	6289
First V	**117**	4	726	174	220	1169	800	770	3339	1753	1069	3302	2039	1137	5412

Group	Ewe #	Days													
		0	**14**	**21**	**70**	**84**	**98**	**112**	**123**	**140**	**154**	**168**	**182**	**196**	**210**
First V	**118**	0	980	132	28	2869	1203	579	4336	2675	2324	5487	4078	3114	8066
First V	**119**	2	1508	165	154	4542	3033	2139	6505	4273	3422	6948	5536	4042	8522
First V	**122**	35	2216	876	1583	6346	4658	3788	2964	2333	3270	7295	4976		13480
First V	**123**	722	6773	4826	2207	5210	3979	4094	6208	4836	4486	7414	511	4580	8794
First V	**124**	23	1993	648	259	2644	1239	837	4755	3306	2030	1577	1258	790	4860
First V	**126**	201	748	229	184	4366	2757	1849	7418	4713	4272	24944	9179	6309	80065
First V	**129**	83	6303	3994	1335	5885	4630	3898	8599	5819	4700	7246	5783	5045	9721
First V	**131**	40	3443	1693	933	3890	3401	2629	5028	3103	2966	5761	4784	3728	13944
First V	**132**	0	2180	734	859	5179	4197	2826	4141	1625	2106	8261	5091	3998	6812
First V	**133**	205	2487	1354	294	5005	3307	3207	6213	6097	4106	10449	7959	6824	9222
First V	**134**	131	4300	2856	597	3716	2416	2261	7273	5503	4435	6845	5496	4422	8537
First V	**139**	103	2264	810	989	5258	4064	3559	5799	4695	3662	6886	6286	5330	9525
First V	**141**	13	4201	2836	258	4768	3222	2317	7674	5452	3982	7621	5888	4276	12100
Control	**128**	1						1585							9
Control	**144**	0						0							0

Group	Ewe #	Days													
		0	**14**	**21**	**70**	**84**	**98**	**112**	**123**	**140**	**154**	**168**	**182**	**196**	**210**
Control	**4**	2						0							1
Control	**15**	1						0							41
Control	**26**	672						34							225
Control	**35**	0						5							33
Control	**64**	290						18							3
Control	**110**	116						67							147
Control	**111**	454						0							2
Control	**112**	77						1							0
Control	**114**	207						87							41
Control	**116**	43						56							20
Control	**120**	1						0							0
Control	**121**	292						1557							1761
Control	**125**	644						1194							1500
Control	**127**	3						176							1143
Control	**130**	3						4							2

Group	Ewe #	Days													
		0	**14**	**21**	**70**	**84**	**98**	**112**	**123**	**140**	**154**	**168**	**182**	**196**	**210**
Control	**135**	72						87							513
Control	**136**	4						3							42
Control	**137**	3						0							4
Control	**138**	70						32							221
Control	**140**	1						40							2
Control	**142**	0						0							1
Control	**143**	0						167							36
Control	**145**	169						276							175

Table A5: Ewe Bodyweights (kg)

Group	Ewe~	Days		
		0	112	210
Prev V	1	47.5	49.5	44.5
Prev V	12	52.0	49.5	42.0
Prev V	23	56.5	50.5	42.0
Prev V	25	61.0	61.0	48.5
Prev V	32	58.0	52.0	49.0
Prev V	33	55.5	51.5	47.5
Prev V	39	59.0	49.5	48.5
Prev V	44	54.5	54.5	46.0
Prev V	48	60.0	54.0	37.5
Prev V	50	61.0	51.0	48.5
Prev V	54	61.5	55.5	51.5
Prev V	56	55.0	54.5	46.5
Prev V	60	63.5	59.0	49.0
Prev V	66	64.0	63.0	53.0
Prev V	68	54.0	45.0	41.5
Prev V	72	55.5	54.0	49.5
Prev V	80	61.0	59.5	52.0
First V	62	63.5	58.0	Dead
First V	78	59.5	59.5	57.5
First V	7	54.5	49.0	41.0
First V	10	55.0	49.0	43.0
First V	13	52.0	48.0	41.5
First V	18	49.0	43.5	39.0

Group	Ewe~	Days		
		0	**112**	**210**
First V	29	57.5	56.0	46.5
First V	37	55.5	49.5	41.5
First V	71	58.5	49.5	41.5
First V	113	48.0	45.0	37.5
First V	115	58.0	51.0	43.5
First V	117	60.0	48.5	41.0
First V	118	52.5	52.5	43.5
First V	119	49.0	48.0	40.5
First V	122	57.5	50.0	49.0
First V	123	49.5	50.0	48.5
First V	124	48.0	49.0	43.0
First V	126	51.5	49.0	43.5
First V	129	61.5	56.0	52.0
First V	131	55.5	49.0	43.5
First V	132	50.5	48.0	45.5
First V	133	53.0	48.0	43.5
First V	134	53.5	58.0	50.0
First V	139	57.0	48.5	48.5
First V	141	44.0	42.0	40.0
Control	128	48.5	49.5	46.0
Control	144	45.0	57.0	53.5
Control	4	63.0	57.5	53.0
Control	15	54.0	55.0	50.0
Control	26	60.0	57.5	55.0

Group	Ewe~	Days		
		0	112	210
Control	35	56.0	52.0	45.0
Control	64	58.0	64.0	56.0
Control	110	61.5	45.0	41.0
Control	111	49.0	41.5	38.5
Control	112	59.5	50.5	42.5
Control	114	58.0	42.0	41.0
Control	116	58.5	50.5	45.0
Control	120	50.5	48.0	46.0
Control	121	55.0	47.0	42.0
Control	125	54.5	50.5	49.0
Control	127	53.0	48.0	46.5
Control	130	56.5	52.0	48.0
Control	135	53.5	55.0	51.0
Control	136	52.0	50.0	42.0
Control	137	52.5	53.0	45.0
Control	138	57.5	57.0	50.5
Control	140	55.5	48.5	42.5
Control	142	52.0	42.0	43.5
Control	143	48.0	48.5	45.0
Control	145	49.0	51.0	48.0

Table A6: Rainfall data provided by the producer (mm)

	Rainfall 2013-14	Average	
September	34	60	
October	18	80	
November	98	133	
December	7	110	
January (14)	55	100	
February	11	94	
March	19	71	(Figures up to 22nd March, there was good rain after that)
TOTAL	**253**	**695**	

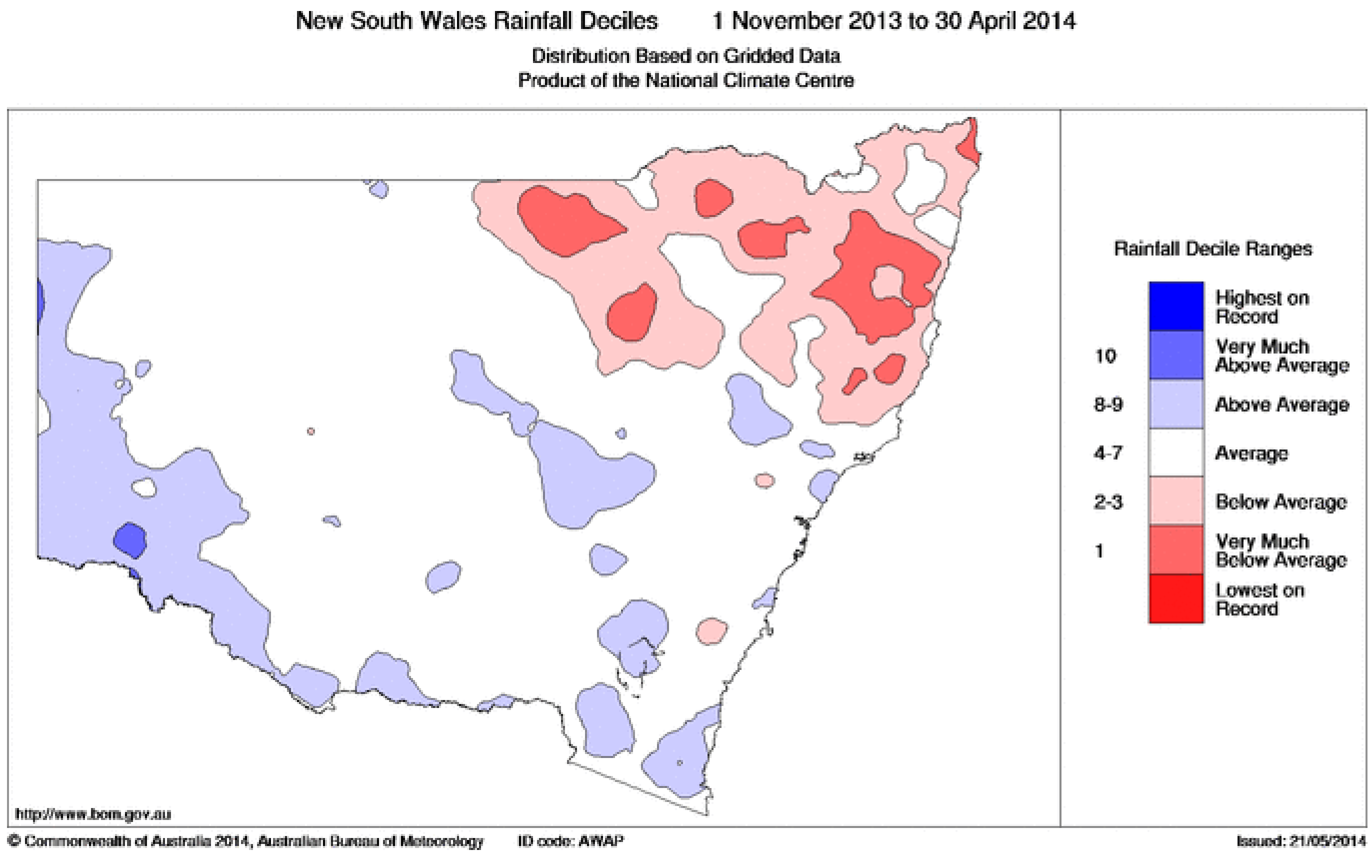


Fig 6. New South Wales Rainfall Deciles between 1 Nov 13 to 30 Apr 14

Appendix 6.3 Kingstown ewe efficacy trial

CH PTY LTD

Study Title: A field study to evaluate the efficacy of an *Haemonchus* vaccine when administered to pre-lambing ewes during times of high parasite challenge. New England district NSW, Australia.

Study No.: **MIHO2918** **Sponsor Study No.:** N/A

Version No.: **5** **FINAL** **Version Date:** 30 June 2014

Author: T. Dale

Sponsor:	Name:	Julie Fitzpatrick Moredun Group Director
	Address:	Moredun Institute The Moredun Group Pentlands Science Park Bush Loan Penicuik Midlothian Scotland, UK
Investigator:	Name:	Tim Dale
	Quals.:	B. LISC
	Address:	Veterinary Health Research Pty Ltd Trevenna Road, Armidale, NSW 2350 Australia

VETERINARY HEALTH RESEARCH PTY LTD

STUDY REPORT

Monitor:	Name:	David Smith
	Address:	The Moredun Group Pentlands Science Park Bush Loan Penicuik Midlothian Scotland, UK

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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: ________________________________

Timothy Dale, B Liv. Sc.

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
13 Aug 13	Study	Protocol V2 – 05 Aug 13	L. Pearson	13 Aug 13
24 Jun 14	Study	Study Report V4 – 11 Jun 14	L. Pearson	30 Jun 14

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:

19. OBJECTIVE

To confirm the field efficacy of a *Haemonchus* vaccine in peri-parturient ewes in the New England region of New South Wales, Australia. Data from this study may be used to support product registration.

20. JUSTIFICATION

Commonly, 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 effective at reducing host anaemia and parasite egg output in lambs and yearlings. This study aims to investigate its efficacy for periparturient and lactating ewes.

21. COMPLIANCE

The study complied with the following national and international standards:

VICH GL9 Good Clinical Practice (issued June 2000)

22. TEST SITE(S)

The trial site location is marked on the site map in Appendix 6.

Animal Phase:	**Laboratory Phase:**
Anonymous	Veterinary Health Research P/L
Uralla NSW 2358	Colin Blumer Animal Health Laboratory
	Trevenna Road
	Armidale NSW 2350 Australia

23. STUDY DATES

Start date (animal phase): 30 JUL 13

Finish date (animal phase): 18 MAR 14

Finish date (laboratory phase): 21 MAY 14

24. STUDY DESIGN

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

h. Animal Model: This study used second lambing Merino ewes on normal pre-lambing prepared paddocks naturally contaminated by *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: Sixty (60) pregnant second lambing ewes were randomly selected from a larger flock, after excessively heavy or light ("outliers", up to ~10% of the flock) animals had been removed. All trial animals were weighed at selection on Day 0 and ranked from heaviest to lightest and sequentially blocked in pairs of two (2) animals. The animals were then randomly allocated into the Group 1 and 2 using the 'draw from hat technique'. Group mean bodyweights at allocation were analysed for significant differences between groups using Student t test and a commercially available software package (Statistix 10.0, 2013). There were no statistical differences ($p<0.05$) between groups.

l. Blinding: Laboratory personnel were blinded to treatment groups when performing faecal egg counts and larval differentiation counts.

25. INVESTIGATIONAL VETERINARY PRODUCT

All formulation details including batch number, expiry date, receipt and usage were recorded on the "Drug Reconciliation" form according to VHR SOP STU-308.

m. Investigational Veterinary Product:

Name:	BarberVax	Batch No.:	08
Composition:	*Haemonchus* antigen and saponin adjuvant	Expiry Date:	01 APR 2015
Dose Level:	5µg antigen and 1mg saponin	WHP:	12 months

n. Source: WormVax Laboratory

Animal Health Laboratory

Dept of Agriculture and Food Western Australia

444 Albany Highway

Albany W.A. 6330

o. Storage: Refrigerated in the Post-Mortem room walk in refrigerator between 2 to 8^{o}C

p. Safety: A MSDS was not provided by the Sponsor (see Deviation #5).

q. Assays: A Certificate of Analysis was provided for the IVP

r. Drug Disposal: The disposal of all remaining IVP will be documented.

26. TREATMENT

Animals in Group 1 were untreated controls, but individual animals in either Group 1 or 2 were treated with a short acting anthelmintic if the following criteria were met:

- ***H. contortus*:** the egg count rose above 10,000 epg or the blood haemoglobin concentration was equal to or fell below 6.5 g/100 mL.
- **Other nematode genera:** (indicated by larval differentiation): the individual animal egg count rose above 1500 epg, or scouring was evident. For a flock treatment, the upper limit was a mean of 1000 epg (though scouring was evident before this level was reached). See NTF #1.
- **Scouring**: Individuals were treated if above an AWI Scour Score of 3.

d. Dose Calculation Dose volume was 1.0 mL IVP by subcutaneous injection. Anthelmintic treatment was calculated according to individual animal bodyweight using Day 0, Day 133 or Day 231 bodyweights.

e. Dose Preparation: The IVP was transported on wet ice bricks and gently shaken immediately prior to the first treatment.

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	Untreated controls	---	---	Anthelmintic treatment occured on Days 0, 28, 189 and 231	30
2	IVP	1mL	Subcut.	Days 0, 28, 91 (marking), 133 (weaning), and 189 Treated with an effective anthelmintic on Days 0 and 231	30

Subcut. = Subcutaneous

27. SCHEDULE OF EVENTS

Table 2: Schedule of Events

Study Day*	Date	Event
Pre-Study		Obtained Animal Ethics Committee approval and received IVP. Confirmed suitable mob of sheep from a commercial sheep farm with *Haemonchus contortus* infection.
0	30 JUL 13	Weighed and tagged (see NTF #5) 60 second lambing Merino ewes and allocated into 2 treatment groups; Group 1 - Untreated Controls and Group 2 – Treat with IVP. Faecal and blood samples were collected from all animals in Group 1 and 2 to conduct a haemoglobin analysis and FECs for group larval differentiation (see NTF #1). All animals in Group 1 and 2 were treated with an effective anthelmintic (CYDECTIN LV + SE - Batch: 1200301, Expiry: JAN 2014). **Group 2 was treated with IVP 'V1'** (see deviation #1 and deviation #2). Processed Group 2 blood samples and harvested Plasma. Group 2 plasma stored in 2 replicates in separate -20^{o}C freezers.

Study Day*	Date	Event
28	27 AUG 13	Faecal and blood samples were collected from all animals in Group 1 and 2 to conduct a haemoglobin analysis and FECs for group larval differentiation (see Deviation #4). Group 1 ewes were treated with anthelmintic (ZOLVIX – Batch: 805523, Expiry: APR 2015). **Group 2 was treated with IVP 'V2'.** Processed Group 2 blood samples and harvested Plasma. Group 2 plasma stored in 2 replicates in separate -20°C freezers.
35	3 SEP 13	Lambing. Commenced twice weekly health observations (see Deviation #6).
91	29 OCT 13	Marking (see Amendment #1 & Deviation #7): Faecal and blood samples were collected from all animals in Group 1 and 2 to conduct a haemoglobin analysis and FECs for group larval differentiation. **Group 2 was treated with IVP 'V3'**. Processed Group 2 blood samples and harvested Plasma. Group 2 plasma stored in 2 replicates in separate -20°C freezers.
105	12 NOV 13	Faecal and blood samples were collected from all animals in Group 1 and 2 to conduct a haemoglobin analysis and FECs for group larval differentiation. Processed Group 2 blood samples and harvested Plasma. Group 2 plasma stored in 2 replicates in separate -20°C freezers.
119	26 NOV 13	Faecal and blood samples were collected from all animals in Group 1 and 2 to conduct a haemoglobin analysis and FECs for group larval differentiation. Processed Group 2 blood samples and harvested Plasma. Group 2 plasma stored in 2 replicates in separate -20°C freezers.
133	10 DEC 13	Weaning (see Deviations #3 & #7): Weighed all animals in Group 1 and 2 and recorded individual bodyweights. Faecal and blood samples were collected from all animals in Group 1 and 2 to conduct a haemoglobin analysis and FECs for group larval differentiation. **Group 2 was treated with IVP 'V4'**. 'Wet and Dry' tested all ewes at sponsors request (See Note to File #2). Processed Group 1 and 2 blood samples and harvested Plasma. Group 1 and 2 plasma stored in 2 replicates in separate -20°C freezers.

Study Day*	Date	Event
147	24 DEC 13	Faecal and blood samples were collected from all animals in Group 1 and 2 to conduct a haemoglobin analysis and FECs for group larval differentiation. Sheep #102 (Group 2) required a salvage drench (ZOLVIX – Batch: 805523, Expiry: APR 2015). Processed Group 2 blood samples and harvested Plasma. Group 2 plasma stored in 2 replicates in separate -20°C freezers.
161	07 JAN 14	Faecal and blood samples were collected from all animals in Group 1 and 2 to conduct a haemoglobin analysis and FECs for group larval differentiation. Processed Group 2 blood samples and harvested Plasma. Group 2 plasma stored in 2 replicates in separate -20°C freezers.
175	21 JAN 14	Faecal and blood samples were collected from all animals in Group 1 and 2 to conduct a haemoglobin analysis and FECs for group larval differentiation. Sheep #326 and #741 (Group 1) required salvage drench (ZOLVIX – Batch: 805523, Expiry: APR 2015). Processed Group 2 blood samples and harvested Plasma. Group 2 plasma stored in 2 replicates in separate -20°C freezers.
189	04 FEB 14	Faecal and blood samples were collected from all animals in Group 1 and 2 to conduct a haemoglobin analysis and FECs for a group larval differentiation. Group 1 ewes were treated with an anthelmintic (ZOLVIX – Batch: 805523, Expiry: APR 2015) see NTF #4. **Group 2 was treated with IVP 'V5'.** Processed Group 2 blood samples and harvested Plasma. Group 2 plasma stored in 2 replicates in separate -20°C freezers.
190	05 FEB 14	Group 1 & 2 Replicate 1 frozen plasma samples (Days 0-175) sent to Moredun Institute.
204	19 FEB 14	Faecal and blood samples were collected from all animals in Group 1 and 2 to conduct a haemoglobin analysis and FECs for group larval differentiation. Sheep #102 and #209 (Group 2) required a salvage drench (QDrench – Batch: F7473, Expiry: AUG 2014). Processed Group 2 blood samples and harvested Plasma. Group 2 plasma stored in 2 replicates in separate -20°C freezers.
217	04 MAR 14	Faecal and blood samples were collected from all animals in Group 1 and 2 to conduct a haemoglobin analysis and FECs for group larval differentiation. Processed Group 2 blood samples and harvested Plasma. Group 2 plasma stored in 2 replicates in separate -20°C freezers.

Study Day*	Date	Event
231	18 MAR 14	Weighed all animals in Group 1 and 2 and recorded individual bodyweights. Blood samples were collected from all animals in Group 1 and 2. Processed Group 1 and 2 blood samples and harvested Plasma. Group 1 and 2 plasma stored in 2 replicates in separate -20^{o}C freezers. All trial animals were treated with an effective anthelmintic. (ZOLVIX – Batch: 805523, Expiry: APR 2015). (See Amendment #2)
	15 Apr 14	Group 1 & 2 Replicate 1 frozen plasma samples (Days 189-231) sent to Moredun Institute.

** In the protocol, amendments, deviations and raw data all activities are timed relative to lambing (Day 0 was 3 SEP 13) but in this report they are timed relative to the day of first vaccination (Day 0 is 30 JUL 13). This is a more accurate way of portraying the kinetics of the trial data because lambing was spread out over a few weeks (see NTF #5).*

28. TEST SYSTEM

Species:	Ovine	**Number:**	60
Breed:	Merino	**Source:**	Commercial sheep farm.
Weight:	30.0 – 42.0 kg (Day 0)	**Health & special requirements:**	Healthy animals. Not within existing WHP and ESI for animal health products used.
Sex:	Second lambing Merino ewes		
Age:	3-4 years	**Method of ID:**	Individually numbered ear tags, coloured group ear tags (see NTF #5).

29. ANIMAL MANAGEMENT

g. Animal Welfare: Study animals were managed similarly and with due regard for their welfare. Animals were monitored twice weekly for health problems according to AEC requirements (see Deviation #6). Animals were handled in compliance with UNE AEC no. 13-107 approved 01AUG13, and any applicable local regulations.

h. Health Management (Concurrent Medications/Salvage Drenches): Study animals were clinically observed at each sampling time-point, no abnormalities were detected during the study.

A salvage drench was administrated to the animals when the individual animal's haemoglobin levels fell below 6.5 g/100mL or had a high scour burden. A summary is provided below:

Day 0; Routine drench, all animals in Groups 1 and 2 were given a salvage treatment.

Day 28; all animals in Group 1 were treated with an effective anthelmintic.

Day 147; animal #102 from Group 2 was treated for low blood haemoglobin content.

Day 175; animals #326 (Group 1) and #741 (Group 1) were treated for low blood haemoglobin content.

Day 189; all animals in Group 1 were treated with an effective anthelmintic (see NTF #4).

Day 204; animals #102 (Group 2) and #209 (Group 2) were treated for low blood haemoglobin content.

Day 231; all trial animals (except for animals #333 and #388; See NTF #3) were drenched out with an effective anthelmintic for the completion of the study.

i. Housing: Routine management practices were followed. All sheep were run as a single mob in the same paddock with *ad lib* to native and improved pastures and a dam for water.

j. Animal Disposal: All ewes used in the trial were returned to commercial herd; not to be sold due to WHP and ESI interval of 12 months from the last treatment date.

30. STUDY PROCEDURES

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

f. Informed Consent: An “Owner Consent and Agreement” form was signed by the Owner and the Investigator post administration of first treatment (see Deviation #1), however, verbal agreement and permission was given prior to first treatment.

g. Weather Data: Data from the nearest Bureau of Meteorology weather station for the study period are included in the raw data (see Appendix 3).

h. Sample Storage, Transfer & Disposal: Sample storage, transfer and disposal were recorded. Replicate 1 plasma samples were dispatched to Moredun Institute for analysis on dry ice via World Courier international dispatch with an accompanying temperature data logger. 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.

31. ASSESSMENT OF EFFECTS

d. Body Weights: Animals were weighed on Days 0, 133 and 231 as 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.

e. Blood Analysis: Single blood samples were collected and recorded from each animal using 18 gauge needles into 8 mL LH Lithium Heparin gel separated Vacuettes 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 & day, sample type, replicate. Frozen plasma samples were forwarded to Moredun Institute for haematology and biochemistry analysis on 05 FEB 14 and 15 APR 14. Key haematological and biochemical parameters were compared to determine treatment effects, if any, and are detailed in the results section of the Study Report.

f. 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. Faecal egg counts and larval differentiation were performed (see Deviation #4). Faecal egg counts and larval differentiation were compared to determine treatment effects, if any, and are detailed in the results section of the Study Report.

32. DATA ANALYSIS

Parasite burdens for each animal were determined from faecal egg counts (see Appendix 5). Percentage efficacy was calculated using the following equation:

[Group Mean (untreated) - Group Mean (treated)] / Group Mean (untreated) x 100

Data from faecal egg counts was entered into Microsoft EXCEL spreadsheet, validated and group arithmetic and geometric means and treatment efficacies were calculated using the spreadsheet.

The total number of individual animal anthelmintic treatments per group was compared.

One-Way Analysis of Variance, its equivalent non-parametric test and additional statistical analysis may be performed as appropriate by the Sponsor's professional statisticians.

33. 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.

34. DATA RECORDS

i. Amendments & Deviations:

Amendment #1: Changed 'Marking' from 01 OCT 13 to 22 OCT 13. The ewes did not start lambing until after Day 0, and the farmer wanted to delay marking until the lambs were ready. This amendment had no impact upon the outcome of the trial.

Amendment #2: The study was concluded after the activities of Day 196 (18 MAR 14) at Sponsor's request. All animals in Groups 1 and 2 were weighed; bloods collected and were treated with an effective anthelmintic. This amendment had no impact upon the outcome of the trial.

Deviation #1: Owner consent was not signed prior to administration of the first treatment. The owner had given verbal consent to use and treat the sheep as he was not able to be at the yards on Day 0. This deviation had no impact upon the outcome of the trial.

Deviation #2: Animals in Group 2 were treated at Day -35 with IVP before the protocol was finalized. Although the Protocol was finalized and approved post Day 0, no changes were made to the final protocol that affected study procedures completed to date. This deviation had no impact upon the outcome of the trial.

Deviation #3: The date of weaning was postponed at the grazier's request because of the poor grazing caused by the drought. This deviation had no impact upon the outcome of the trial.

Deviation #4: On Day 28 (27 AUG 13) the FEC samples were pooled into a single culture, instead of being cultured by groups. There was a misunderstanding between the study investigator and diagnostic lab staff. Sponsor was notified post event. Every sample since has been cultured by Group. This deviation had no impact upon the outcome of the trial.

Deviation #5: The Sponsor did not provide an MSDS for the IVP. Indeed, it was not deemed essential for pilot batches of the vaccine. This deviation had no impact upon the outcome of the trial.

Deviation #6: The Record of Animal Care was misplaced. The grazier gave the investigator verbal confirmation that the livestock were checked twice weekly (sometimes more) for health observations through his normal management/ animal husbandry practices. This deviation had no impact upon the study as no animal was excluded from the study and no adverse events or abnormalities were detected either by the grazier or VHR staff.

Deviation #7:

a) Deviation to Amendment #1. The day 'Marking' actually occurred on 29 OCT 13 (Day 56).
b) Clarification of terminology in Amendment #1; the term 'Marking' related not just to the date but also to all the activities that were to be conducted on that occasion as outlined in the protocol.
c) Clarification of terminology in Deviation #3; the term 'Weaning' related not just to the date but also to all the activities that were to be conducted on that occasion as outlined in the protocol, i.e. V4 was given on 10 DEC 13 (Day 98).

j. Notes to File:

Note to File #1: If the non *Haemonchus* egg count of an individual sheep, (calculated from the total egg count and the Coproculture data) exceeded 1,500 epg that sheep was drenched at the next sampling date.

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.

Note to File #2: All ewes were "wet and dry" tested at the sponsor's request. 'Wet' ewes were defined as ewes which still contained milk in the udder as they are rearing a lamb. 'Dry' ewes were defined as ewes which did not contain any milk in the udder, due to the loss of lamb. Dry sheep ID's were #199, #363, #417, #270, #294, #356, #107, #521, #405, #510, #741, #769 and #279.

Note to File #3: On 23 FEB 14, Kingstown was hit by a storm and strong winds caused a tree to fall over a fence line allowing the trial animals to merge with a different mob. Grazier Jamie Swales stated that he would draft the ewes back into their rightful mob for each trial. Only 58 out of the 60 animals for trial MIHO2918 were returned to their paddock. Animals #333 and #388 were missing for the last 2 visits; Day 217 and Day 231. Therefore no data was collected from these two animals during the last visits.

Note to File #4: On Day 189 the Controls were treated with Zolvix as per the protocol. This treatment should not have occurred but unfortunately its presence in the Protocol was overlooked by the trial monitor. It prevented further useful comparison between the groups and so the trial was ended earlier than planned.

Note to File #5:

a) All sheep enrolled in the trial had the same coloured ear tag (orange) with unique individual ID number. All animals in Group 2 (IVP) were given a second plain orange tag to differentiate between groups. The unique ID tag number was always checked against each animal before any activity was conducted, eg: before vaccinating, all animals had their ID checked prior to administrating the vaccine.
b) In the protocol, amendments, deviations and raw data all activities are timed relative to lambing (Day 0 was 3 SEP 13) but in this report they are timed relative to the day of first vaccination (Day 0 is 30 JUL 13). This is a more accurate way of portraying the kinetics of the trial data because lambing was spread out over a few weeks

k. Change of Study Personnel: There were no changes in study personnel over the duration of the study.

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 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.

35. RESULTS

i. **Lambing success and number of ewes used in the study:** There were sixty (60) ewes at the start of the trial but eight (8) in the Vaccinated and five (5) in the Control groups, did not raise a lamb, either because they were not pregnant, or their lamb was born dead or died soon after birth. These thirteen (13) sheep remained with the rest of the flock throughout the trial but their data was excluded from the analysis.

ii. **Types of comparison made:** It was of particular interest to know how well the vaccine performed during the "periparturient" phase of the trial because worm eggs shed during lactation are an important source of infection for the next generation of lambs and hence the general epidemiology of Haemonchosis in a flock. Therefore the degree of protection attained from lambing to weaning is presented in addition to that calculated for the whole duration of the trial.

iii. **Rainfall and pasture infectivity:** The 2013-2014 summer was exceptionally dry in New England and the trial site was no exception, especially during December and January (Appendix 3, Table A1).

iv. ***Haemonchus* Egg Counts:** During lactation *Haemonchus* egg counts were significantly reduced in the Vaccinated sheep compared to the Controls (Fig 1, Table 3, p<0.01) and the same was true over the whole period of the trial (Fig 2, Table 3, p<0.001).

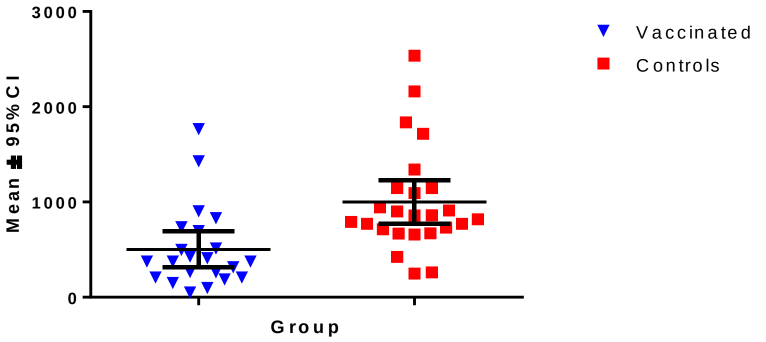

Fig 1. Individual ewe egg counts averaged from lambing to weaning

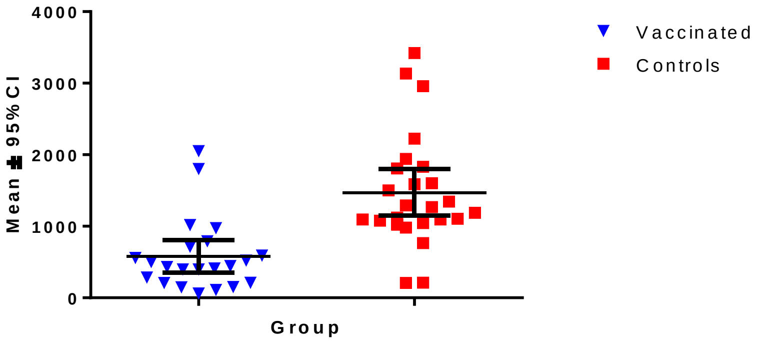

Fig 2. Individual ewe egg counts averaged over the whole trial

Thus BarberVax reduced *Haemonchus* egg output by 60.6% over the course of the whole trial and by 49.5% during the lactation phase, respectively.

Table 3: Percent protection (eggs) on each sampling day

Days	**Mean *Haemonchus* epg**		**%Prot**	**P value**
after V1	**Control**	**Vaccine**		**(t-test)**
0	344	311	**9.7**	n/s
28	60	28	**53.7**	n/s
91	850	994	**-17.0**	n/s
105	691	327	**52.7**	***
119	895	458	**48.8**	***
133	692	426	**38.5**	*
147	925	358	**61.3**	***
161	1940	475	**75.5**	****
175	3880	1171	**69.8**	****
189	1947	454	**76.7**	****

n/s: non-significant; * P<0.05; ** P<0.02; *** P<0.01; **** P<0.001;

v. **Kinetics of and relationships between the parameters studied over the course of the trial:** Antibody titres in the unvaccinated control ewes remained at background levels close to zero throughout the trial (Fig 3 and Appendix 3, Table A4). Group mean titres in the Vaccinated ewes also remained low until two weeks after their third immunization at marking time, when they rose to a temporary peak of around 7,700. A similar pattern was observed after each subsequent vaccine boost, which is a sharp increase in titre followed by a somewhat slower decline, so that antibody concentrations gradually increased as the trial progressed (Fig 3).

Total and *Haemonchus* specific group mean faecal egg counts were very similar within each group throughout the trial, reflecting the fact that *Haemonchus* was always the dominant gastrointestinal nematode genus infecting the ewes (Fig 3 and Appendix 3, Tables A2 and A3).

Mean Vaccinate and Control *Haemonchus* specific counts were similar up until marking time when they both approached 1000 epg (Table 3), but after that those in the vaccinates were significantly lower until Day 204, two weeks after anthelmintic had mistakenly been given to the Control group. During January the Controls showed a big increase in egg output, but this was substantially suppressed in the Vaccinated ewes (Fig 3).

Mean blood haemoglobin concentrations in the Vaccinated ewes remained relatively steady during the trial fluctuating between 9 and 10 g/100mL (Appendix 3, Table A5). Control values were similar except for a noticeable dip during their egg count peak in January and February when they were significantly lower than Vaccinates, (Fig 4). Seven lactating ewes required a precautionary drench during the course of the trial, four Controls and two Vaccinates, one of which was treated twice (as per Table A2). In five cases this was because the blood haemoglobin had fallen below the 6.5 g/100mL threshold and in two cases because egg counts exceeded 10,000 per g. These drenches were given, when egg counts were at or close to their maximum (Table A2).

Despite the dry conditions and delayed weaning, the ewes put on weight during the trial (Fig 4 and Appendix 3, Table A6). There was no discernible difference between the groups, but the final weighing took place 6 weeks after the Controls had been mistakenly given a drench, a factor which may have confounded that result.

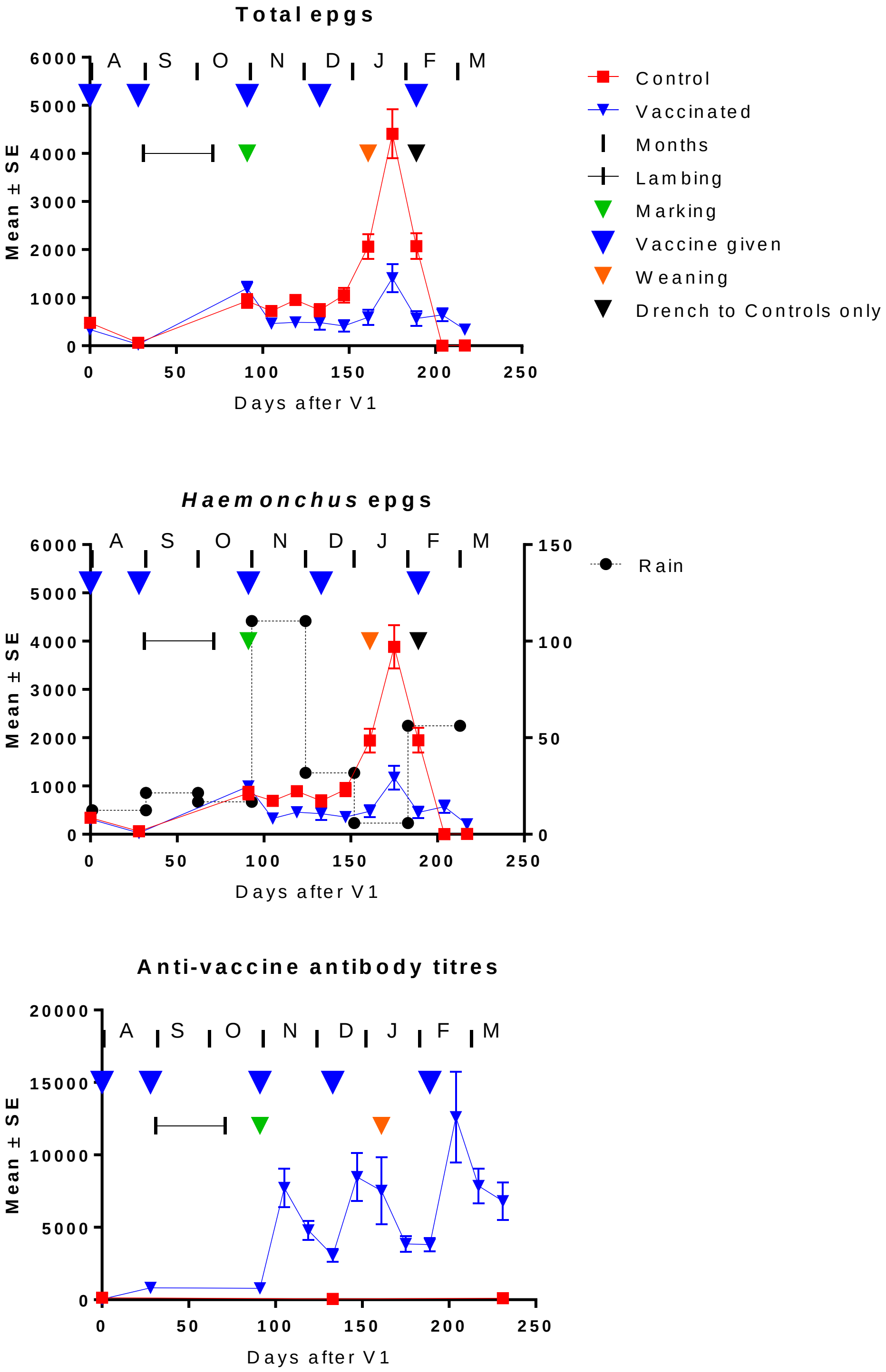

Fig 3. Kinetics of interventions, group mean total egg counts, *Haemonchus* specific egg counts and the anti-vaccine antibody response in lactating ewes.

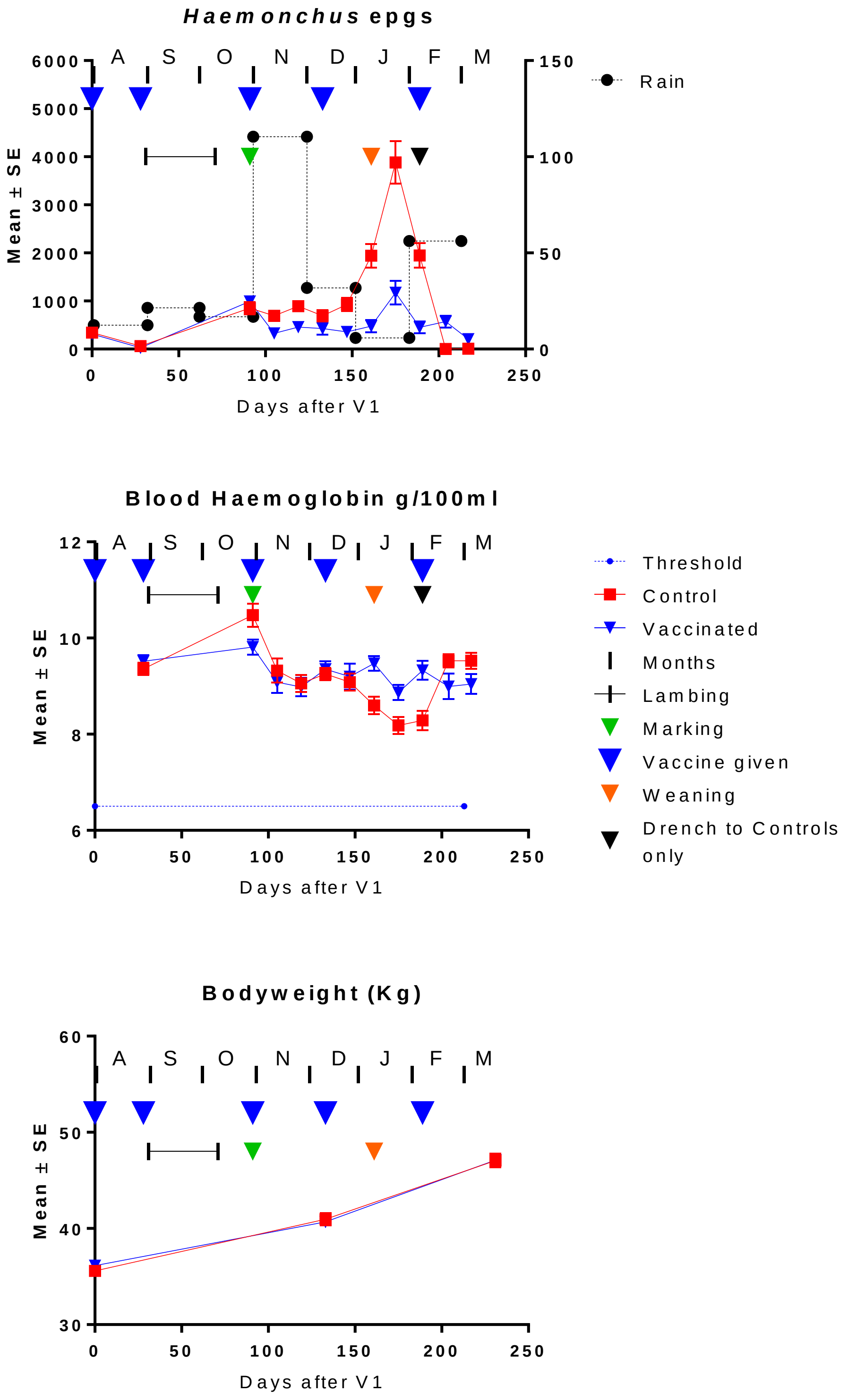

Fig 4. Kinetics of the *Haemonchus* specific egg counts in relation to blood haemoglobin concentrations and to bodyweight in lactating ewes.

36. CONCLUDING REMARKS

It was clear that the course of BarberVax given during the trial, starting with two vaccinations pre-lambing (one of which could coincide with shearing on some properties) followed by further injections at lamb marking, weaning and later in the summer, effectively suppressed Barber's Pole worm egg output by the ewes and most of the associated anemia.

This difference was apparent both during the phase when the ewes had lambs at foot and post weaning when the risk of Haemonchosis is usually at its highest. Thus by suppressing the "periparturient rise", the vaccine offers a method for reducing pasture infectivity for the next generation of lambs, thus curbing the buildup of infection over the high risk summer "season". Although several vaccinations were needed to achieve this, most could be fitted in with other management practices, reducing the number of special musters needed. Furthermore the two parallel trials have shown that, if the ewes had been vaccinated in a previous season the protective effect was stronger and one less vaccination was needed.

37. REFERENCES

Nil.

APPENDIX 3

WEATHER DATA

Table A1. From Kingstown Post Office – Australian Meteorological Bureau data

Rainfall (mm)

	Aug	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**
2013-2014	12.4	21.4	16.8	110.4	31.8	5.8	56.2	100.4
Mean	39.8	47.5	63.8	88.6	95	89.3	82.3	50.9

Weather Data Cont.

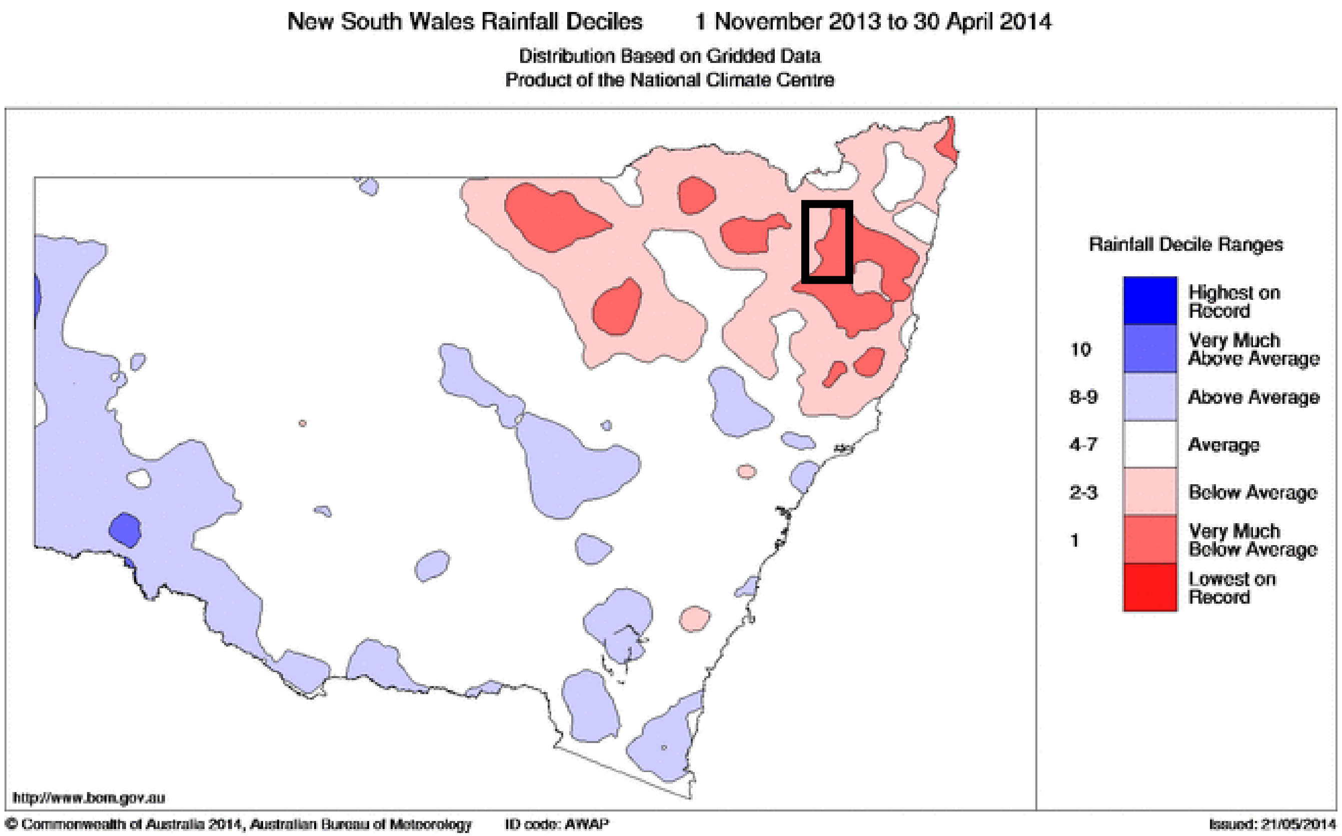

Figure 5. New South Wales rainfall deciles with trial site location marked.

APPENDIX 4

TABULATED RAW DATA

Table A2. Faecal egg counts (EPG)

epg>10K, drench next sampling

HB<6.5 precautionary drench

Dry= ewes which did not rear a lamb and therefore excluded from the calculations

Lambing from days 35 to 70 after V1

V1 V2 V3 V4 V5

Days after V1

Ewe#		Group	Dry	0	28	91	105	119	133	147	161	175	189	204	217
356		Control	Y	1920	40	1560	1640	4360	2280	2600	2680	5920	6160	40	0
363		Control	Y	320	0	120	200	240	120	440	2240	4760	5120	0	0
510		Control	Y	520	80	760	720	680	1040	1520	2720	4000	2400	0	0
521		Control	Y	560	360	520	720	1920	1080	2320	2360	4680	3400	0	0
741		Control	Y	200	0	600	1720	320	280	480	640	1200	40	40	0
1		Control		0	0	1520	320	760	280	960	3240	5520	3640	0	0
2		Control		1360	320	3480	2720	440	2960	2680	4160	6760	5520	0	0
43		Control		840	0	680	360	1000	600	960	1960	3800	5040	0	0
98		Control		560	40	760	600	880	1440	880	2840	2800	5320	0	0
123		Control		720	80	1280	600	720	1040	1120	1160	2760	3720	0	0

Ewe#		Group	Dry	0	28	91	105	119	133	147	161	175	189	204	217
145		Control		80	0	680	680	1000	280	400	1280	3360	3840	0	0
161		Control		640	280	720	760	600	600	880	1560	2880	2200	0	0
311		Control		200	80	360	760	1000	400	840	2440	5800	0	0	0
326		Control		640	80	400	360	1040	760	520	1880	3320	560	0	0
329		Control		480	0	1080	1480	1320	160	200	1800	6080	4240	0	0
333		Control		760	0	800	520	1440	1040	920	3920	3320	3440	0	NS
348		Control		920	80	960	880	2240	1000	2160	3880	11040	6000		0
375		Control		200	NS	440	760	1120	400	920	1080	4680	4440	0	NS
388		Control		760	80	880	480	1320	520	1520	2720	6920	9280	0	NS
420		Control		160	40	280	520	560	120	1360	1800	5440	6640	0	0
443		Control		360	0	1160	1120	1480	1120	3560	5600	10400	9120		0
508		Control		720	0	200	280	240	80	800	40	160	1560	0	40
514		Control		1080	160	2200	1440	2160	2080	1600	2400	5160	5520	0	80
524		Control		200	120	440	680	1120	880	960	1480	2600	3400	0	0
658		Control		120	0	760	440	800	720	840	1440	3160	4160	0	0
662		Control		80	0	1640	240	400	120	520	1360	3560	3280	0	0
669		Control		80	40	320	440	400	480	0	40	80	40	0	0
671		Control		520	80	1040	800	560	560	480	880	3440	3600	0	0

Ewe#		Group	Dry	0	28	91	105	119	133	147	161	175	189	204	217
830		Control		400	0	960	520	640	680	1000	1520	4320	5720	0	0
846		Control		80	0	320	240	560	280	200	1120	2880	4680	0	40
107		IVP	Y	240	120	1760	200	280	200	320	120	280	360	120	40
199		IVP	Y	1480	120	1520	600	600	600	800	640	1360	2040	1120	520
270		IVP	Y	320	0	1520	1080	2040	1360	1240	1280	3520	3600	2560	1920
279		IVP	Y	240	40	1360	1240	1560	1040	200	200	1600	2440	1720	440
294		IVP	Y	400	0	760	1720	1000	520	1240	1880	2760	2240	1560	360
405		IVP	Y	560	40	1120	760	NS	760	440	120	2160	2160	1960	80
417		IVP	Y	160	0	0	0	40	80	0	80	40	80	200	40
769		IVP	Y	80	40	760	680	1240	800	360	320	1520	2000	520	400
32		IVP		80	0	1320	360	480	880	560	1680	2760	2000	200	40
78		IVP		560	40	1640	240	120	80	NS	200	1120	2160	80	0
102		IVP		520	NS	440	240	280	200	80	0	0	NS	40	0
143		IVP		0	0	120	80	80	40	0	40	200	80	40	0
178		IVP		400	40	720	160	160	200	40	80	520	800	80	0
205		IVP		120	0	440	80	0	80	40	80	280	960	200	0
209		IVP		840	40	1120	280	160	320	200	920	920	2080	240	NS
218		IVP		280	0	800	120	120	40	200	640	2400	3600	1160	800

Ewe#		Group	Dry	0	28	91	105	119	133	147	161	175	189	204	217
241		IVP		200	40	1360	200	360	280	240	240	1280	1200	360	280
261		IVP		280	0	2360	240	480	160	160	160	80	80	120	40
300		IVP		1080	120	1600	480	280	160	240	360	1840	5120	640	360
327		IVP		0	0	640	520	640	320	120	0	440	2440	400	200
365		IVP		520	0	1960	1520	1160	1800	1680	2160	4720	6960	2160	40
380		IVP		320	80	1440	2080	2240	2480	2120	2240	4640	3920	2240	800
427		IVP		80	0	560	200	0	40	200	120	240	280	240	40
447		IVP		200	40	920	80	160	120	200	440	1520	2440	1600	1000
504		IVP		120	0	1280	400	560	NS	0	0	1040	440	400	360
591		IVP		120	0	2360	640	1120	0	680	1160	2160	1240	960	440
638		IVP		920	40	640	480	280	320	520	1520	1640	2400	720	240
643		IVP		40	40	1040	160	80	80	80	80	440	1280	680	360
716		IVP		360	80	1480	960	1320	1680	760	160	920	840	1040	1720
749		IVP		400	40	2120	600	640	880	520	200	440	320	560	360

Table A3. **Coproculture data: Percent of each nematode genus identified**

Group	Day	Treatment	Haem.	Trich.	Ost.	Coop.	Oes.	Total
1	0	Control	72%	19%	7%		2%	100%
2		IVP	92%	4%	4%			100%
1	28	1 & 2	98%		2%			100%
2								
1	91	Control	91%	4%	5%			100%
2		IVP	83%	7%	10%			100%
1	105	Control	96%	1%	3%			100%
2		IVP	71%	15%	14%			100%
1	119	Control	94%	2%	4%			100%
2		IVP	94%	3%	3%			100%
1	133	Control	93%	2%	5%			100%
2		IVP	88%	4%	7%		1%	100%
1	147	Control	88%	3%	9%			100%
2		IVP	87%	11%	2%			100%
1	161	Control	94%	2%	4%			100%
2		IVP	80%	12%	7%		1%	100%

1	175	Control	98%		2%			100%
2		IVP	74%	15%	11%			100%
1	189	Control	99%				1%	100%
2		IVP	94%	3%	3%			100%
1	204	Control	100%					100%
2		IVP	89%	7%	3%		1%	100%
1	217	Control	100%					100%
2		IVP	62%	26%	12%			100%

Table A4. ELISA titres

Days after V1

Group	Ewe #	Lamb	0	28	91	105	119	133	147	161	175	189	204	217	231
V	107	no	18	173	599	7147	5576	5164	4136	3459	2825	2633	7064	5447	5105
V	199	no	8	554	2316	54205	22337	13756	14501	13670	9037	8373	68867	36884	21183
V	270	no	8	7	165	2600	1325	576	3120	2426	1532	1965	4180	3209	2811
V	279	no	0	1368	947	3747	2113	1187	3098	3376	1495	1759	4984	3966	2987
V	294	no	2	34	43	5089	4506	3009	5215	4327	2802	3018	11300	7258	5877
V	405	no	0	587	2318	7267	4914	3641	6883	6469	5276	4869	8306	6227	5815
V	417	no	280	76	699	6880	5248	3796	7185	6526	5509	5161	9739	8611	6359
V	769	no	38	1056	1506	2883	2034	1670	7962	5699	4787	4417	9690	6367	5127
V	32		0	156	207	6028	3129	1851	1629	1418	932	1055	6528	4623	4920
V	78		4	73	289	5739	4649	3730	5954	4489	3668	4128	13571	6871	6300
V	102		21	387	1662	7979	5202	4084	6628	5909	3743	4093	10310	6514	5954
V	143		94	1238	1706	9694	5594	4296	11718	8053	5650	4711	12213	9756	7533
V	178		7	2567	132	5139	3109	1348	7987	5398	2836	2603	9866	6397	4938
V	205		141	1530	1181	8607	4412	2562	5104	4046	2727	2436	8114	5764	4325

Group	Ewe #	Lamb	0	28	91	105	119	133	147	161	175	189	204	217	231
V	209		1	42	918	5979	3940	2222	1830	1283	947	1497	5429	3242	3127
V	218		2	157	27	7375	4558	2492	3475	2967	1762	1879	4944	3376	2436
V	241		187	654	954	6817	5323	3714	9316	7555	6014	5405	11546	7858	7189
V	261		20	1956	2104	28260	14401	9038	25134	51901	10738	9265	19939	13610	10991
V	300		17	71	234	5233	4558	1219	4970	4212	2147	1808	4271	3544	2427
V	327		61	624	1266	6019	4389	3460	6695	6081	5484	5547	16668	12435	7383
V	365		5	2195	219	4498	2732	1063	480	733	731	1101	5257	3264	2583
V	380		389	29	251	449	468	311	3072	2305	1356	1671	4901	3830	2891
V	427		48	3481	1593	21752	11795	7209	34030	22271	9618	7652	74681	28825	13303
V	447		97	690	1092	8617	5556	3639	8965	5567	4383	4636	10286	8118	6418
V	504		32	40	477	5273	3840	2308	8279	5123	3958	3334	7836	6818	5355
V	591		0	645	1169	8680	4966	3390	4480	3593	2727	3057	7820	6494	4871
V	638		84	0	1	7350	5100	3287	2907	2307	1798	2310	7562	6210	5029
V	643		5	592	1250	8123	5917	4401	9261	5488	4664	4430	8747	6503	6183
V	716			43	538	1646	1099	880	8374	6142	4527	5452	6369	5194	4402
V	749			927	306	670	805	828	15987	8813	4613	5878	20635	13693	31277
C	356	no	292					237							373

Group	Ewe #	Lamb	0	28	91	105	119	133	147	161	175	189	204	217	231
C	363	no	78					108							97
C	510	no	0					0							0
C	521	no	20					54							88
C	741	no	1					0							1
C	1		121					64							321
C	2		0					6							2
C	43		0					1							0
C	98		95					78							283
C	123		11					162							151
C	145		65					24							147
C	161		7					2							22
C	311		289					133							82
C	326		8					20							10
C	329		38					34							28
C	333		6					15							
C	348		50					33							133
C	375		23					21							80

Group	Ewe #	Lamb	0	28	91	105	119	133	147	161	175	189	204	217	231
C	388		64					31							0
C	420		18					18							82
C	443		48					31							125
C	508		1					0							15
C	514		2					2							5
C	524		0					0							0
C	658		48					24							43
C	662		14					21							41
C	669		552					344							418
C	671		2130					410							409
C	830		45					25							8
C	846		31					20							10

Table A5. Blood Haemoglobin (g/100ml)

Group	Ewe#	Lamb	Days after V1										
			28	91	105	119	133	147	161	175	189	204	217
C	356	no	9.7	7.6	9.2	8.1	9.6	8.7	8.7	8.8	8.7	9.1	9.8
C	363	no	9.3	13.1	12.5	11.8	9.6	8.0	9.2	9.5	9.3	10.8	9.8
C	510	no	9.5	11.4	8.5	8.4	8.4	8.3	7.7	7.8	8.1	9	9.4
C	741	no	9.5	11.7	12.0	9.7	9.8	9.2	9.8	4.8	7.7	9.5	9.3
C	1		9.5	11.2	12.0	9.7	9.0	8.6	6.9	7.6	7.4	8.7	8.4
C	2		9.7	7.4	7.2	7.4	8.7	8.9	8.5	7.4	7	9.5	9
C	43		10.2	11.9	9	10.4	10.5	10.4	10.0	9.0	9.2	10	9.8
C	98		9.4	9.4	10.9	9.3	9.2	9.0	8.8	8.4	8.4	9.7	7.1
C	123		9.6	11.7	8.4	9.2	9.7	11.6	9.4	9.0	9.2	9.9	10.2
C	145		9.6	11.6	11.3	9.7	9.8	8.2	8.7	8.1	8.7	10	9.8
C	161		8.9	11.0	7.7	9.2	9.1	10.4	8.8	8.1	8.2	8.8	9.5
C	311		9.1	11.2	10.0	8.8	8.7	8.5	7.2	7.1	7.1	8.3	9.3
C	326		7.8	9.0	8.6	8.1	8.2	7.5	7.5	6.5	8.3	8.6	9
C	329		9.6	8.5	8.5	7.9	8.9	9.4	8.4	8.5	8.1	9.7	9.7
C	333		10.0	9.1	9.5	8.1	9.4	8.5	7.3	6.6	7	9	NS
C	348		8.5	10.7	10.5	9.0	9.5	9.1	7.8	7.8	7.6	10	10.6
C	375		9.1	10.1	8.1	9.6	8.9	9.4	8.8	8.1	8.4	9.7	9.9
C	388		9.7	10.8	10.5	8.9	9.7	9.3	8.2	7.7	8.6	10.8	NS
C	420		8.9	12.7	8.8	9.8	10.3	9.3	8.9	8.1	7.7	9.6	9.9
C	443		8.9	10.6	9.4	7.9	8.9	8.5	7.7	9.4	6.5	9	8.8
C	508		9.0	11.5	8.6	10.0	9.8	9.5	9.4	9.7	9.9	9.8	10.4
C	514		9.6	9.2	8.7	7.8	8.4	8.4	8.9	8.3	8.4	10.2	10.1
C	521		7.7	9.2	7.8	8.1	8.3	8.0	6.7	6.9	6.6	7.9	8.6
C	524		8.4	10.4	9.4	9.3	8.8	9.1	9.4	8.8	8.8	9.8	9.7

Group	Ewe#	Lamb	Days after V1										
			28	91	105	119	133	147	161	175	189	204	217
C	658		10.1	10.5	8.2	8.6	9.4	8.8	10.0	8.8	8.7	9.6	10.3
C	662		9.8	11.7	11.1	10.4	9.5	9.2	8.8	8.4	7.6	9.4	9.2
C	669		10.8	10.8	10.4	10.7	10.6	10.2	10.4	10.3	10.7	11.1	10.2
C	671		9.5	10.9	7.7	9.4	8.5	8.5	8.5	8.1	8.1	8.9	8.7
C	830		9.0	9.4	8.4	8.2	8.8	8.8	9.0	7.5	10	8.7	9.3
C	846		9.2	10.7	10.2	9.0	9.0	8.0	7.6	7.3	7.6	9.4	10.2
V	107	no	9.6	10.2	10.4	10.2	10.6	9.3	9.8	9.6	10.7	10.9	10
V	199	no	8.9	6.9	10.3	7.1	9.8	9.6	9.3	7.0	7	8.3	8.5
V	270	no	9.8	10.6	10.1	9.7	9.7	9.8	10.1	9.9	9.1	9.3	8.6
V	279	no	9.1	9.9	9.3	9.5	9.6	9.5	10.4	9.8	10.9	10	10.6
V	294	no	9.1	9.2	9.5	8.9	9.3	9.1	9.0	8.5	8.8	9	8.2
V	405	no	9.0	9.0	8.9	8.9	9.7	9.7	9.1	9.2	9.2	8.7	9.6
V	417	no	9.1	10.0	9.6	9.4	9.5	8.7	9.8	9.4	9.2	9.9	9.1
V	769	no	8.3	8.1	8.2	7.7	8.5	8.7	8.6	8.8	8.4	8.4	8.9
V	32		9.0	9.6	9.3	9.1	9.2	9.5	9.2	9.2	8.9	9.4	9.6
V	78		10.0	11.0	8.7	10.2	10.4	9.5	10.4	8.9	9.8	9	9.6
V	102		8.7	9.5	9.3	7.4	8.2	4.5	8.2	9.0	8.2	6.3	7.6
V	143		10.2	10.7	10.2	10.0	10.2	9.8	9.9	9.7	10.2	10	6.7
V	178		9.8	9.7	9.7	9.8	9.0	9.0	8.7	8.4	9.5	9.4	9.1
V	205		9.9	10.0	9.1	10.2	9.2	9.0	9.9	9.3	9.7	9.3	9.2
V	209		8.7	9.3	9.3	8.8	8.5	9.8	8.5	8.2	8.5	5.3	8.5
V	218		8.9	11.7	11.7	10.3	10.0	10.8	10.1	10.3	9.2	10.1	9.3
V	241		8.3	9.7	9.8	9.0	9.4	8.9	9.1	9.4	9.8	9.3	8.9
V	261		9.9	10.6	7.9	8.8	10.0	9.6	9.9	10.0	9.8	10.3	9.9
V	300		10.3	9.6	10.4	9.3	8.7	9.4	9.5	8.2	9.1	8.9	9.4
V	327		9.1	10.3	10.0	9.7	8.5	10.2	9.7	9.6	9.7	9.6	8.8

Group	Ewe#	Lamb	Days after V1										
			28	**91**	**105**	**119**	**133**	**147**	**161**	**175**	**189**	**204**	**217**
V	365		10.0	9.5	8.7	8.0	10.3	8.3	8.9	7.7	8	8.2	9.5
V	380		10.3	9.4	8.1	8.2	9.0	9.2	9.1	7.7	7.4	7.5	9.8
V	427		9.2	9.7	10.2	8.9	9.3	8.7	8.9	7.7	8.7	9.5	8.1
V	447		10.5	10.0	8.7	9.5	10.8	10.9	9.4	8.6	9.1	9.7	9.1
V	504		9.3	9.2	7	9.7	10.1	10.5	10.4	9.2	11.3	8.9	8.5
V	591		8.8	8.7	7.6	8.1	8.0	8.3	9.3	9.2	10.6	9.3	8.5
V	638		9.3	9.3	7.6	8.8	8.4	8.3	8.7	8.3	9.1	8.4	7.5
V	643		9.0	10.9	9.5	9.5	9.3	10.4	10.7	10.2	11	11.3	9.7
V	716		9.7	8.7	8.7	8.3	8.8	8.0	8.6	8.4	9.9	8.5	11.5
V	749		10.0	9.3	8.7	7.5	9.9	9.5	10.4	8.4	8.5	9.3	9.6

Table A6. Ewes bodyweight (kg)

Group	Ewe#	Lamb	0	133	231
			Days after V1		
Control	356	no	35.5	40	46
Control	363	no	34.5	47	52.5
Control	510	no	33	37.5	45.5
Control	521	no	35.5	38	42.5
Control	741	no	38.5	44.5	48
Control	1		33.5	34	39
Control	2		35.5	42	48.5
Control	43		34	44.5	42
Control	98		33.5	39.5	47
Control	123		38.5	43.5	53
Control	145		31.5	36.5	42
Control	161		33.5	37.5	44.5
Control	311		38	41.5	48
Control	326		34	37	47.5
Control	329		33	39.5	46
Control	333		37	43	-
Control	348		39	43.5	46.5
Control	375		35	38.5	44
Control	388		35.5	41.5	-
Control	420		37	38.5	50.5
Control	443		34	39.5	46.5
Control	508		40	44.5	50
Control	514		33	38.5	46
Control	524		36.5	40.5	48
Control	643		35	43	52

Control	662		33	40.5	47
Control	669		36.5	43	45.5
Control	671		39	42	48.5
Control	830		36.5	44.5	49
Control	846		37.5	47.5	51.5
Vaccine	107	no	34	47	52
Vaccine	199	no	35.5	45	51.5
Vaccine	270	no	34	41.5	47
Vaccine	279	no	34	41.5	48.5
Vaccine	294	no	30	40.5	44.5
Vaccine	405	no	38.5	44.5	52
Vaccine	417	no	36.5	47.5	53.5
Vaccine	769	no	35.5	42	47.5
Vaccine	32		33	37	45.5
Vaccine	78		33.5	39	44.5
Vaccine	102		38	39.5	47.5
Vaccine	143		42	43.5	50
Vaccine	178		39	44.5	49
Vaccine	205		38	40	44
Vaccine	209		37.5	41.5	48.5
Vaccine	218		36.5	42.5	53
Vaccine	241		39	46.5	53

Days after V1

Group	Ewe#	Lamb	0	133	231
Vaccine	261		37	42.5	50.5
Vaccine	300		35	37	40.5
Vaccine	327		38.5	43.5	48.5
Vaccine	365		33	41.5	47.5

Vaccine	380		36	43	47.5
Vaccine	427		34	38	45
Vaccine	447		36.5	39	45.5
Vaccine	504		37.5	41.5	46
Vaccine	591		34.5	38	43.5
Vaccine	638		33	38	46.5
Vaccine	638		38	40.5	48
Vaccine	716		32	38	44
Vaccine	749		33.5	40.5	48.5

Appendix 6.4 Large scale safety trial

VETERINARY HEALTH RESEARCH PTY LTD

STUDY REPORT

Study Title: A field study to evaluate the safety under field use conditions of an *Haemonchus* vaccine when administered subcutaneously post-weaning to ewes during times of high parasite challenge. New England district NSW, Australia.

Study No.: **MIHO2937** **Sponsor Study No.:** N/A

Version No.: **3** **Version Date:** 24 March 2014

Author: T. Dale

Sponsor:	Name:	Julie Fitzpatrick Moredun Group Director
	Address:	Moredun Institute The Moredun Group Pentlands Science Park Bush Loan Penicuik Midlothian Scotland, UK

Appendix 6.4 Large scale safety trial

VETERINARY HEALTH RESEARCH PTY LTD

STUDY REPORT

Sponsor Monitor & Representative:	Name: Address:	David Smith The Moredun Group Pentlands Science Park Bush Loan Penicuik Midlothian Scotland, UK
Investigator:	Name: Quals.: Address:	Tim Dale B. LISC Veterinary Health Research Pty Ltd Trevenna Road, Armidale, NSW 2350 Australia

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38. OBJECTIVE

A field study to evaluate the safety under field use conditions of an *Haemonchus* vaccine when administered subcutaneously post-weaning to ewes during times of high parasite challenge. Data from this study may be used to support product registration.

39. JUSTIFICATION

Commonly, 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 too 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 effective at reducing host anaemia and parasite egg output. This study aims to confirm the safety when breeding ewes are vaccinated according to both 'label directions' (single dose) and twice "label directions" (two doses) on each of two occasions.

40. 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 Apr 07)

41. TEST SITE

Animal Phase:

Anonymous

Uralla NSW 2358

Australia

Laboratory Phase:

Veterinary Health Research P/L

Colin Blumer Animal Health Laboratory

Trevenna Road

Armidale NSW 2350 Australia

42. STUDY DATES

Start date (animal phase): 17 Feb 14

Finish date (animal phase): 21 Mar 14

Finish date (laboratory phase): N/A

43. STUDY DESIGN

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

n. Animal Model: This study used second lambing Merino ewes due to their on-property retention for the full anticipated 12 month withhold period. Study ewes were grazed upon normal pre-weaning prepared paddocks with likely contamination by *Haemonchus contortus*.

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

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

q. Allocation: Cohort 1 animals: Seventy (70) second lambing Merino ewes post-crutching were randomly selected as they presented in the animal handling facility from a larger flock of approximately 600 animals. All 70 selected animals were individually identified (eartag), weighed, and ranked by bodyweight. The 70 animal's bodyweights were graphed and excessively heavy or light "outliers" (the 5 highest bodyweights and lightest 5 bodyweights) were removed from the allocation. The 10 excluded animals were reintegrated into Cohort 2. The remaining 60 animals were sequentially blocked into twenty (20) blocks, each of 3 animals. 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 10.0, 2013) and can be found in Appendix #6.

Cohort 2 animals: Remaining animals were given either of two different coloured tags, orange or purple. Group 1 (270+) animals received an Orange tag and Group 2 (270) animals received a Purple tag.

r. Blinding: Not applicable

44. INVESTIGATIONAL & CONTROL PRODUCTS

s. Investigational Veterinary Product:

Name:	BarberVax	Batch No.:	09
Composition:	*Haemonchus* antigen and saponin adjuvant	Expiry Date:	01 Apr 15
Dose Level:	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 in the PM Room walk in refrigerator between 2 to 8^{o}C (See Appendix 7 datalogger temperature).

v. Safety: A MSDS was not provided by the Sponsor (See Deviation #1).

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

x. Drug Disposal: The balance of remaining vaccine was given to sheep grazier Jamie Swales.

45. TREATMENT

a. Dose Calculation:

Cohort 1 Group 1 animals (20) were retained as untreated control animals

Group 2 animals (20) were vaccinated on each of two occasions 4 weeks apart with a single dose of IVP.

Group 3 animals (20) were vaccinated on each of two occasions 4 weeks apart with two doses of IVP.

Cohort 2 Group 1 animals (270+) were vaccinated on each of two occasions 4 weeks apart with a single dose of IVP.

Group 2 animals (270) were vaccinated on each of two occasions 4 weeks apart with two doses of IVP.

b. Dose Preparation: Dose volume was 1.0 mL IVP by one subcutaneous injection (single dose) or 2.0 mL by subcutaneous injection (double dose) given as two injections of 1.0 mL at two different sites a minimum of 5 cm apart.

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

Table 1: Treatment Regime

Cohort	Grp.	IVP Details	Dose Level	Dose Volume	Route	Freq.	Trt. Day(s)	No. Anim.
1	1	Untreated	-	-	-	-	-	20
1	2	BarberVax	1.0 mL per Animal	1 x 1.0 mL	Subcut. Right neck	2	Days 0 and 28	20
1	3	BarberVax	1.0 mL per Animal	2 x 1.0 mL	Subcut. Right neck	2	Days 0 and 28	20
2	1	BarberVax	1.0 mL per Animal	1 x 1.0 mL	Subcut. Right neck	2	Days 0 and 28	270+
2	2	BarberVax	1 mL per Animal	2 x 1.0 mL	Subcut. Right neck	2	Days 0 and 28	270

All animals were treated using either a Simcro Vaccine Gun or NJ Phillips Vaccine Gun at a dose level of 1.0 mL subcutaneously, using an 18 gauge ½ inch vaccination needle. Study animals were observed immediately post treatment, no abnormalities were observed.

46.<u>SCHEDULE OF EVENTS</u>

Table 2: Schedule of Events

Study Day	**Date**	**Event**
Pre-study	-	Obtained Animal Ethics Committee approval. Confirmed suitable groups of sheep on selected commercial sheep farm.
17 Feb 14	-1	Weighed, tagged, monitored body temperatures and conducted clinical observations on 70 (Cohort 1) animals. Allocated animals into Groups as per protocol. No animal required a more detailed clinical examination.
18 Feb 14	0	Recorded body temperature from all animals in Cohort 1. Conducted clinical observations on Cohorts 1 and 2. No animal required a more detailed clinical examination. Treated all animals with IVP as per their group.

19 Feb 14	1	Recorded body temperature from all animals in Cohort 1. Conducted clinical observations on Cohorts 1 and 2. No animal required a clinical examination.
20 Feb 14	2	Recorded body temperature from all animals in Cohort 1. Conducted clinical observations on Cohorts 1 and 2. No animal required a clinical examination.
21 Feb 14	3	Recorded body temperature from all animals in Cohort 1. Conducted clinical observations on Cohorts 1 and 2. No animal required a clinical examination.
17 Mar 14	27	Weighed and recorded body temperature on all animals in Cohort 1, Groups 1, 2 and 3. Conducted clinical observations on Cohorts 1 and 2. No animal required a more detailed clinical examination.
18 Mar 14	28	Recorded temperature from all animals in Cohort 1. Conducted clinical observations on Cohorts 1 and 2. No animal required a more detailed clinical examination. Treated all animals with IVP as per their group.
19 Mar 14	29	Recorded temperature from all animals in Cohort 1. Treated Conducted clinical observations on Cohorts 1 and 2. No animal required a more detailed clinical examination.
20 Mar 14	30	Recorded temperature from all animals in Cohort 1. Treated Conducted clinical observations on Cohorts 1 and 2. No animal required a more detailed clinical examination.
21 Mar 14	31	Weighed and recorded body temperature on all animals in Cohort 1, Groups 1, 2 and 3. Conducted clinical observations on Cohorts 1 and 2. No animal required a more detailed clinical examination.

47. TEST SYSTEM

Species:	Ovine	**Number:**	600
Breed:	Merino	**Source:**	Commercial sheep farm
Weight:	39.5 – 51.5 kg (D.31 bwt)	**Health & special requirements:**	Healthy Animals
Sex:	Second lambing Merino ewes		
Age:	4 years	**Method of ID:**	Cohort 1: Unique ID tag Cohort 2: Coloured ear tag

48. ANIMAL MANAGEMENT

k. 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. AEC13-016 approved 01AUG13, and any applicable local regulations.

l. Health Management: No health problems or adverse events were observed during the study.

m. Housing: Routine management practices were followed. Study animals in Cohort 1, Groups 1, 2 and 3 were grazed as a discreet single group in paddocks of native and improved pasture. Cohort 2, Groups 1 and 2 were grazed as a discrete single group in paddocks of native and improved pasture (separate from Cohort 1).

n. Animal Disposal: Animals treated with the IVP will not enter the human food chain for 12 months past the last treatment with the IVP on Day 28. An "Animal Accountability" form was completed.

49. STUDY PROCEDURES

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

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

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

50. ASSESSMENT OF EFFECTS

g. 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.

h. Clinical observations: All animals were observed in a group paddock setting on Days -1, 0, 1, 2, 3 thence twice weekly to Day 26 thence Days 27, 28, 29, 30 and 31 according to VHR SOP FLD-409 and recorded on a "Clinical Observations Record".

i. Clinical Examinations: No clinical examination was conducted as no trial animal showed any signs and symptoms of abnormal behavior or ill effects towards the vaccine.

j. Body Temperatures: Body (rectal) temperatures were recorded at intervals outlined in section 9 - Schedule of Events. Rectal temperatures during the study were compared between groups to determine treatment effects, if any, and are detailed in the results section of the Study Report.)

51. STATISTICAL ANALYSIS

Data from body temperature and bodyweight was entered into a computer spreadsheet (Microsoft EXCEL); validated and group arithmetic means calculated using the spreadsheet.

One-Way Analysis of Variance, its equivalent non-parametric test and / or additional statistical analysis may be performed as appropriate by the Sponsor's professional statisticians.

52. 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.

53. DATA RECORDS

q. Amendments & Deviations:

Deviation #1: The sponsor did not provide an MSDS or Certificate of analysis of the IVP 'BarberVax', which was not deemed essential for pilot batches '09' of the vaccine. This deviation had no impact upon the outcome of the trial.

r. Notes to File: There were no notes to file.

s. Change of Study Personnel: There were no changes of personnel during the trial.

t. 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 were paginated prior to appending to the final Study Report.

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

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

w. 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.

x. Study Report: The original signed Study Report with raw data 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.

54. RESULTS

a) Bodyweights

Group mean and standard error bodyweights are plotted in Fig 1 and individual values are presented in Table 3.

All three groups gained weight during the trial. Analysis of variance did not detect any differences between the groups except on day 31, when the group vaccinated with the double dose of Barbervax were significantly lighter than the controls (see Appendix 6 for detailed statistical output)

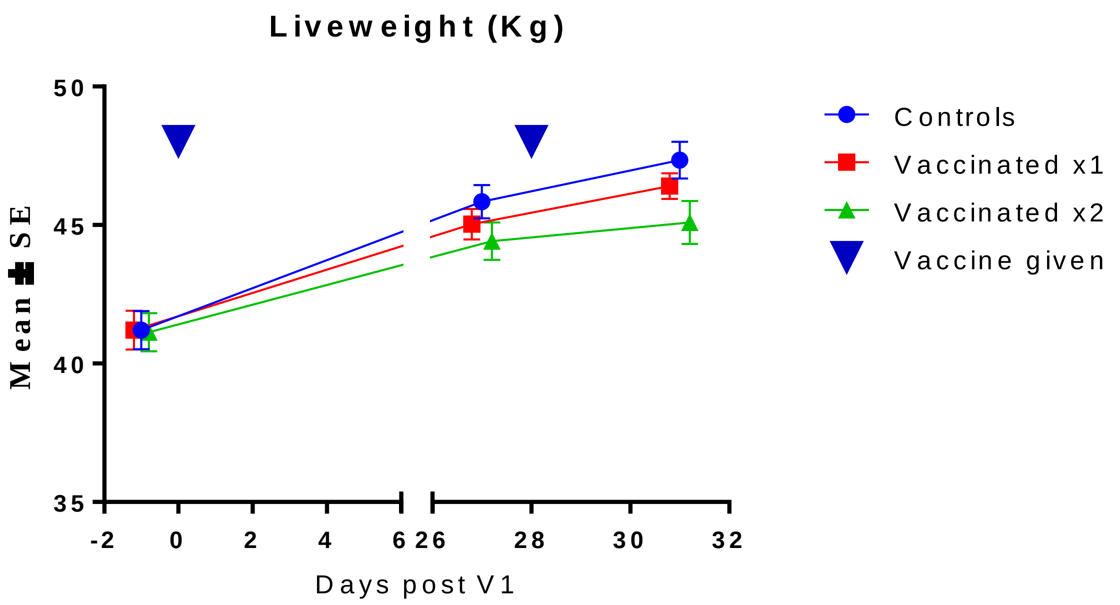


Fig 1. Group mean and standard error bodyweights.

Table 3. Individual, group mean and standard error bodyweights (kg) on the day before and 27 and 31 days after the sheep were first vaccinated.

Group 1				Group 2				Group 3			
	Day after V1										
Tag no	-1	27	31	Tag no	-1	27	31	Tag no	-1	27	31
8331	41.0	48.0	50.5	8335	42.0	50.5	48.5	8332	38.0	42.5	44.5
8333	44.5	48.5	51.5	8337	41.5	45.5	45.0	8334	41.5	44.0	45.0
8338	39.0	42.0	43.5	8339	43.5	47.0	48.0	8336	40.5	43.5	45.5
8343	45.5	47.0	48.5	8340	35.5	43.5	46.0	8345	37.5	41.5	40.5
8347	37.5	43.5	46.5	8341	46.0	47.5	50.0	8346	40.0	41.5	40.5
8349	39.5	42.0	43.0	8342	38.0	42.0	44.0	8348	39.0	42.0	42.0
8350	41.5	48.5	51.0	8344	39.0	44.0	44.0	8351	42.5	44.0	47.0
8352	39.0	45.0	46.5	8360	36.5	41.5	44.0	8353	46.0	49.5	50.0
8356	38.0	48.5	45.0	8362	45.0	47.0	47.5	8358	41.5	42.5	43.5
8361	46.0	48.5	51.5	8363	39.5	43.5	43.5	8359	43.5	47.5	50.0
8365	42.0	47.0	49.0	8374	42.0	46.5	50.0	8366	43.0	43.0	47.0
8369	36.0	42.0	42.5	8378	45.5	47.5	46.5	8368	41.0	47.5	46.5
8371	42.5	47.5	48.5	8382	43.5	45.0	47.0	8370	36.0	43.5	42.5
8372	46.0	49.5	51.0	8386	40.5	43.0	44.5	8380	38.0	44.5	44.0
8373	44.0	46.0	47.0	8387	38.0	42.0	43.5	8381	46.0	46.0	45.0
8376	41.0	46.0	49.5	8389	43.0	46.0	47.5	8384	44.5	50.5	51.0
8377	42.0	47.5	47.5	8391	46.0	48.5	49.0	8392	39.5	41.5	41.0
8379	43.5	46.0	46.0	8395	40.0	44.0	45.5	8393	45.5	48.5	49.5
8390	39.5	43.5	45.5	8397	37.5	42.0	47.0	8394	42.5	46.0	47.5
8396	36.0	40.5	43.0	8398	41.5	44.0	47.0	8400	36.5	39.0	39.5

Mean	41.2	45.9	47.4	41.2	45.0	46.4	41.1	44.4	45.1
SE	0.698	0.605	0.67	0.712	0.56	0.47	0.689	0.68	0.78

b) Clinical observations:

The results are recorded in Table 4. No signs of abnormal behaviour were observed in any sheep during the trial.

Table 4. Clinical observations after vaccination with Barbervax on Day 0 and 28

(NAD = no abnormality detected)

		Days after first vaccinated									
Sheep	**Group**	**-1**	**0**	**1**	**2**	**3**	**27**	**28**	**29**	**30**	**31**
8331	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8333	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8338	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8343	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8347	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8349	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8350	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8352	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8356	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8361	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8365	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8369	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8371	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8372	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8373	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8376	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8377	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD

8379	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8390	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8396	1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8335	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8337	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8339	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8340	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8341	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8342	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8344	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8360	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8362	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8363	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8374	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8378	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8382	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8386	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8387	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8389	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8391	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8395	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8397	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8398	2	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8332	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8334	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8336	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8345	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8346	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD

8348	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8351	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8353	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8358	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8359	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8366	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8368	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8370	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8380	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8381	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8384	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8392	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8393	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8394	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8400	3	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD

A single animal from Cohort 2, Group 1 died on March 12 (Day 25 after V1) of suspected myiasis as there was extensive fly-strike damage over the animal

c) Clinical Examinations:

None were made because no trial animal showed any signs and symptoms of abnormal behavior or ill effects towards the vaccine.

d) Body Temperatures:

Individual, group mean and standard error rectal temperatures are presented

in Table 5 and the last two of these parameters are plotted in Fig 2. (Note that for reasons of clarity the data plotted in Fig 2 has been slightly offset along the X-axis).

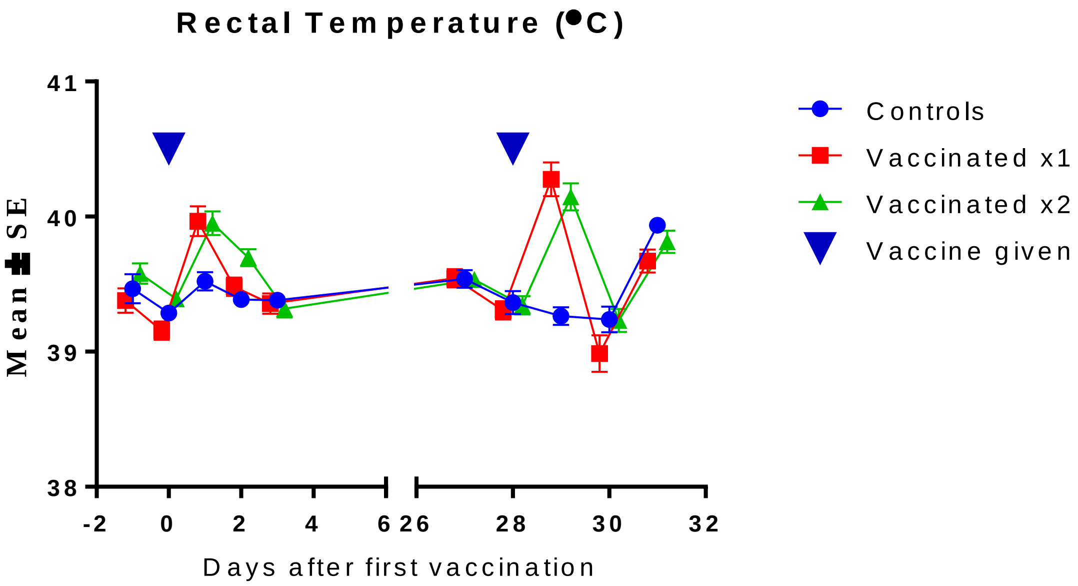


Fig 2. Group mean and standard error rectal temperatures.

Table 5. Individual, group mean and standard error body temperatures

		Days after first vaccination									
Tag	**Group**	**-1**	**0**	**1**	**2**	**3**	**27**	**28**	**29**	**30**	**31**
8331	1	39.2	39.3	39.1	39.4	39.1	39.1	39.4	39.3	39.5	39.5
8333	1	39.1	39.3	38.9	39.1	39.3	39.3	39.8	39.4	39.4	40.2
8338	1	40.1	39.9	40.1	39.3	39.3	39.7	39.0	38.9	39.3	39.3
8343	1	39.5	39.6	39.5	39.3	39.3	39.6	39.9	39.4	38.6	39.9
8347	1	39.6	39.2	39.4	39.4	39.5	39.5	38.4	39.4	39.2	39.9
8349	1	39.3	39.3	39.7	39.5	39.6	39.8	39.4	39.3	39.4	39.9
8350	1	39.4	39.6	39.3	39.5	39.5	39.9	39.9	39.4	39.8	40.3
8352	1	39.6	39.0	39.5	39.0	39.4	39.9	39.5	39.1	39.1	40.2

8356	1	38.9	38.9	39.4	39.2	39.5	39.4	39.5	39.2	39.3	39.9
8361	1	39.1	39.3	39.6	39.2	39.6	39.3	39.5	39.4	38.3	40.3
8365	1	39.7	39.3	39.5	39.3	39.1	39.3	38.6	38.9	39.1	39.7
8369	1	39.3	39.2	39.6	39.6	39.2	39.8	39.6	39.3	39.1	40.0
8371	1	40.0	39.7	39.5	39.4	39.5	39.4	39.1	39.8	40.1	40.3
8372	1	39.0	39.3	39.4	39.2	39.2	40.0	39.6	39.3	39.2	39.9
8373	1	39.5	39.2	39.7	39.7	39.9	39.8	39.6	39.3	39.3	40.0
8376	1	38.2	39.2	39.6	39.3	39.2	39.2	39.2	38.9	38.4	39.7
8377	1	40.3	39.0	39.3	39.7	39.7	39.2	39.6	39.4	39.8	39.8
8379	1	40.0	39.1	40.2	39.8	39.1	39.7	39.1	39.9	39.3	40.0
8390	1	40.0	38.9	39.2	39.4	39.3	39.8	39.2	39.1	39.3	40.0
8396	1	39.5	39.4	39.9	39.4	39.3	39.1	39.4	38.6	39.3	39.9
Mean		**39.5**	**39.3**	**39.5**	**39.4**	**39.4**	**39.5**	**39.4**	**39.3**	**39.2**	**39.9**
SE		0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.1
		-1	**0**	**1**	**2**	**3**	**27**	**28**	**29**	**30**	**31**
8335	2	39.2	39.2	39.6	39.6	39.0	39.2	39.7	41.0	39.6	39.7
8337	2	39.6	39.3	40.6	40.0	39.7	39.2	39.7	40.9	39.8	39.8
8339	2	38.3	39.8	40.4	40.0	39.7	39.8	39.2	40.7	39.8	40.0
8340	2	39.2	38.9	39.6	39.2	39.0	39.5	39.9	40.4	39.5	39.9
8341	2	39.0	38.8	39.3	39.4	39.5	39.6	39.0	40.3	38.2	39.2
8342	2	39.5	39.1	40.8	39.1	40.0	39.7	39.3	40.9	39.5	39.7
8344	2	39.2	39.0	39.6	39.0	39.4	39.6	39.3	40.1	38.6	39.8
8360	2	39.1	39.2	40.0	39.4	39.1	39.8	39.6	40.3	38.6	39.9
8362	2	39.5	39.3	39.6	39.6	39.2	39.7	39.3	39.9	39.2	38.6
8363	2	39.2	39.2	39.6	39.7	39.1	39.2	39.4	39.6	38.7	39.5
8374	2	39.7	39.6	40.8	39.7	39.5	39.7	39.3	39.2	38.1	40.0
8378	2	40.3	39.3	39.8	39.5	39.4	39.3	39.4	40.9	38.3	40.3
8382	2	39.2	38.4	40.9	39.3	38.6	40.1	39.2	40.8	39.6	40.2

8386	2	39.4	39.4	39.5	39.8	39.5	40.0	39.5	40.1	39.2	39.9
8387	2	39.4	39.2	39.5	39.3	39.1	39.3	38.8	40.0	38.9	39.2
8389	2	39.6	39.2	40.2	39.5	39.7	39.1	39.2	40.8	39.4	39.5
8391	2	39.1	39.2	39.7	39.3	39.1	39.1	38.5	39.2	39.2	39.7
8395	2	39.5	39.2	39.8	38.9	39.2	39.9	39.2	40.0	38.9	39.6
8397	2	39.6	38.7	39.7	39.9	39.9	39.3	39.3	39.7	37.6	39.4
8398	2	40.0	39.1	40.3	39.4	39.4	39.8	39.3	40.7	39.0	39.5
Mean		**39.4**	**39.2**	**40.0**	**39.5**	**39.4**	**39.5**	**39.3**	**40.3**	**39.0**	**39.7**
SE		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
8332	3	39.5	39.1	40.4	39.4	39.4	39.1	39.3	41.0	39.0	39.3
8334	3	39.6	39.6	40.0	39.8	39.6	39.6	39.4	40.4	39.7	39.9
8336	3	39.7	39.4	40.8	39.6	39.4	39.4	39.7	40.5	39.1	39.7
8345	3	39.1	39.1	39.7	39.7	39.1	39.6	39.0	39.9	39.2	39.6
8346	3	39.6	39.5	40.0	39.6	39.4	39.3	39.4	40.7	39.7	39.8
8348	3	39.6	39.3	40.0	39.7	39.4	39.6	39.6	39.4	39.1	39.7
8351	3	39.6	39.3	39.3	39.7	39.6	39.4	39.5	39.8	38.5	40.2
8353	3	39.7	39.2	39.9	39.9	39.4	39.4	39.0	40.6	39.5	39.4
8358	3	39.6	39.8	39.7	39.8	39.4	39.7	39.8	40.0	39.2	39.9
8359	3	39.6	39.2	40.2	39.4	39.4	39.5	38.9	40.2	38.7	39.9
8366	3	40.1	39.5	40.1	40.1	39.5	39.2	39.3	40.2	39.1	39.8
8368	3	39.4	39.5	39.6	39.6	39.2	39.9	39.4	40.5	38.7	39.1
8370	3	38.8	39.4	40.6	39.4	38.8	39.8	39.3	40.5	39.6	40.0
8380	3	40.0	39.8	39.5	39.9	39.4	39.4	39.1	39.8	39.0	39.9
8381	3	38.8	39.2	39.5	39.9	38.9	39.6	39.0	40.2	39.7	40.5
8384	3	39.8	39.5	40.5	40.5	39.9	39.7	39.0	40.0	39.5	39.8
8392	3	39.8	39.4	39.6	39.5	39.3	40.0	40.1	39.4	39.3	40.5
8393	3	39.6	39.5	40.0	39.3	39.4	39.6	39.3	40.5	39.5	39.5

8394	3	39.8	39.2	39.7	39.7	39.3	39.6	39.5	39.8	39.8	40.3
8400	3	39.9	39.3	39.9	39.4	38.6	39.3	39.3	39.5	38.7	39.5
Mean		**39.6**	**39.4**	**40.0**	**39.7**	**39.3**	**39.5**	**39.3**	**40.1**	**39.2**	**39.8**
SE		0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Rectal temperatures were significantly higher in both vaccinated groups compared to the controls one day after each vaccination (Fig 2, Table5 and Appendix 7 for ANOVA results).

CONCLUSIONS

It was concluded that vaccination caused a temporary pyrexia a day later. On average this rise in body temperature was less than one degree Centigrade and lasted for only one day. The result was the same irrespective of whether one or two vaccinations had been administered and was insufficient to give rise to any detectable changes in behaviour.

Despite this, by four days after the second vaccination, the group vaccinated with the double dose of vaccine did not gain weight as fast as the other two groups. Possibly this was a consequence of reduced appetite and hence lower herbage intake associated with the temporary pyrexia detected two days earlier, but, if so it would be expected in both vaccinated groups, since a similar degree of pyrexia was recorded in each.

The overall conclusion was that the adverse signs associated with administration of Barbervax were mild and commercially acceptable. The data essentially confirmed that of the earlier, smaller scale trial in housed sheep (see Appendix 8-6.1) and broadly agreed with published descriptions of the side effects of other ruminant vaccines containing saponin (see Appendix 8.3).

Appendix 6.5. Extensively raised lamb trial

Introduction.

A vaccine trial was conducted with extensively raised lambs on a property in the North West plains of NSW. Producers in this region would be reluctant to adopt the 5 vaccination schedule recommended for Barbervax in New England lambs because the effort and expense of mustering on their more extensively grazed properties would be prohibitive. Currently these farmers largely control Barbers Pole by giving a long acting drench, usually closantel or moxidectin, at weaning. However, properties with worms resistant to these drugs are becoming more common and so the possibility that control could be achieved by giving Barbervax at marking and weaning followed by subsequent boosts at 6 week intervals was investigated.

The trial was conducted by Dr F. Fishpool, who had recently completed a PhD on gastrointestinal nematodes in sheep. She was under the direction of S. Slattery and L. Guest, veterinarians based at the LHPA office at Narrabri.

Design of the trial

This trial was conducted on a private property some 20 kms west of Narrabri.

One hundred and twenty Merino lambs were randomly allocated to three groups treated as follows:- 1) vaccinated at marking and weaning, 2) given moxidectin (Weanergard) at weaning or 3) untreated controls. Fifty animals were assigned to each treatment group and 20 to the controls. All grazed the same paddock with their mothers.

Faeces were sampled at marking on 28 October when the lambs were 2 months old, then 3 weeks later, at weaning on January 6, and 14 and 45 days post weaning. Egg counts were made by Veterinary Health Research in Armidale. Blood samples for serology were collected at marking, weaning and 2 and 4 weeks later. The haemoglobin concentrations of the samples collected at marking, weaning and 2 weeks post weaning were also determined. The serology was done at the Moredun Research Institute using a standard ELISA which detected the antibody response to the vaccine antigens. Blood haemoglobin was measured on the farm using a Haemacue device.

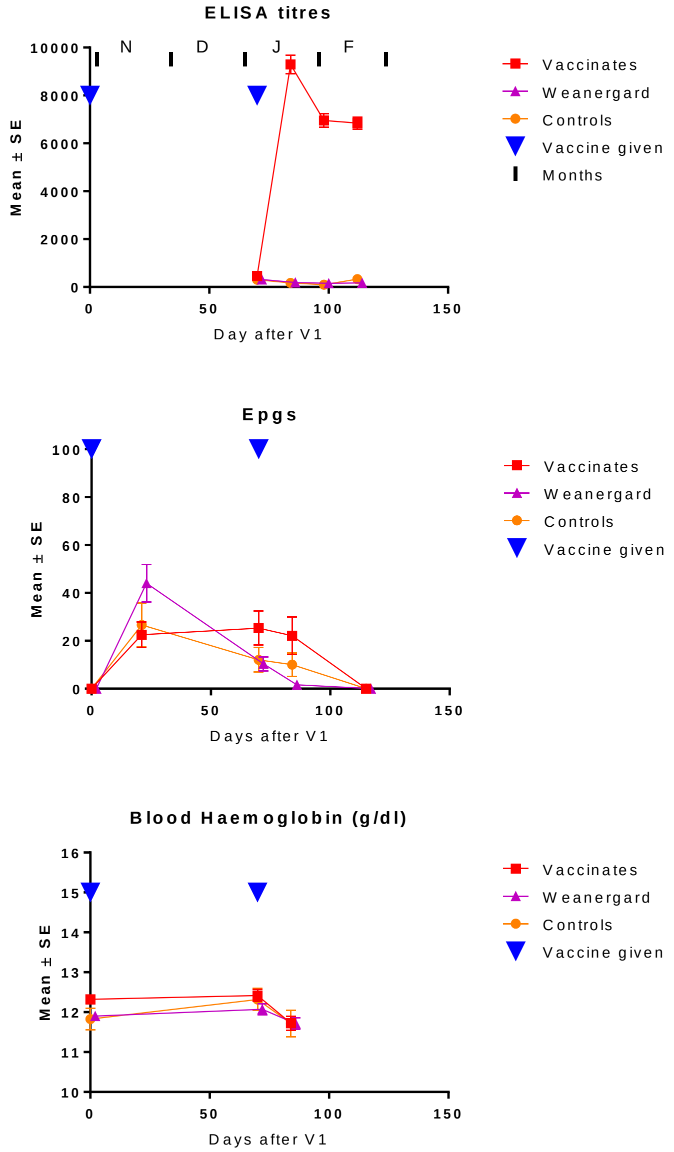
Results

These are summarised in the graphs of Fig. 1. Mean egg counts in all three groups were always less than 50 eggs per g throughout the trial, with no obvious difference between the groups.

Blood haemoglobin concentrations remained at normal concentrations throughout the trial, no differences were detected between the groups.

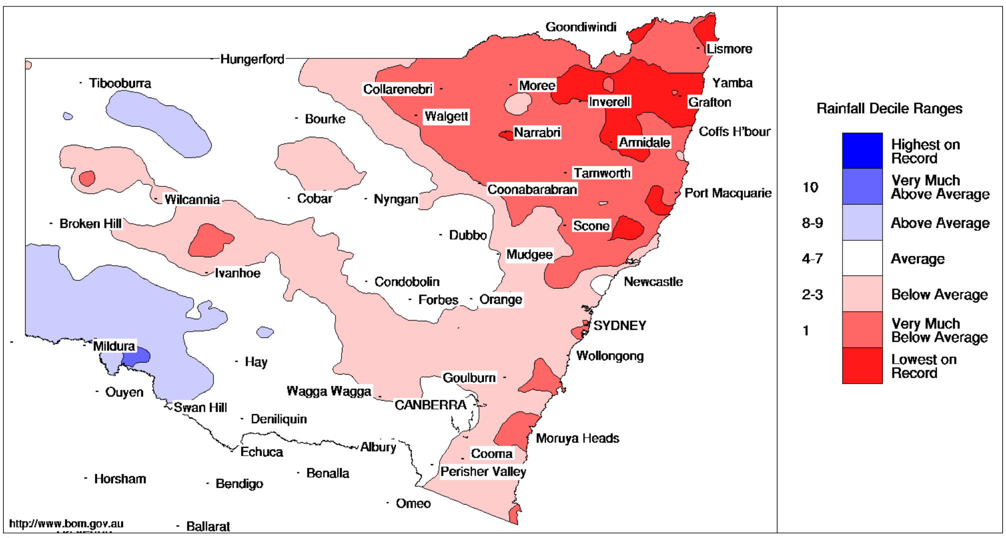
Elisa titres were at baseline levels in all three groups until weaning and remained so in the moxidectin and control groups until the end of the trial. In contrast, antibody concentrations rose sharply two weeks after the vaccinates received their boost at weaning. Mean titres rose to about 9,000 before falling off to approximately 7,000 for the rest of the study.

Fig 1. Kinetics of antibodies, egg counts and haemoglobin



New South Wales Rainfall Deciles 1 December 2013 to 28 February 2014

Distribution Based on Gridded Data
Product of the National Climate Centre



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Discussion

It was unfortunate that the exceptionally dry summer (see rainfall map below), resulted in insufficient natural *Haemonchus* challenge to determine directly whether the vaccine could have afforded any useful protection.

Nevertheless, it was useful to know that a boost of Barbervax given 10 weeks after a primary vaccination stimulated a response very similar to that obtained in earlier New England trials where the interval between the first two immunisations was only 3 or 4 weeks (Fig 2).

It seems highly likely that the response stimulated by the boost given at weaning would have been protective, because lambs on two New England properties possessed similar titres after their first or second boost and their egg counts were significantly reduced compared to unvaccinated controls (Fig 2).

Conclusion

The results were inconclusive, because natural challenge of the vaccine did not occur.