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Barbervax, a vaccine for *Haemonchus contortus* infection of sheep: attempts to extend the registration claim to include goats

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Abstract

Barber's Pole Worm (*Haemonchus contortus*) is an important parasite of goats and sheep in Australia and overseas. Control relies on anthelmintic drugs combined with pasture management, but strains of *Haemonchus* resistant to these drugs are common and widespread. Compared to sheep producers, goat farmers have relatively few options to control gastrointestinal nematode parasites because many anthelmintics are registered for sheep only.

Barbervax, a vaccine for Barber's Pole Worm, has recently been registered for use in Australian sheep. Preliminary and on-going trials overseas suggest that Barbervax could work in goats.

Three efficacy field trials with kids were performed in the Northern Tablelands of NSW with a view to obtaining caprine registration in Australia. Unfortunately the results were mixed: one trial worked well, a second showed some positive effects, but a third failed. Because the anti-vaccine antibody responses were similar in all three trials, the underlying cause of the variable vaccine efficacy is not understood.

It was concluded that the results were too variable for registration to be granted by the regulators.

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1 Background

Barbervax, a novel vaccine for the Barber's Pole Worm (*Haemonchus contortus*), was first registered for use in Australian lambs in 2014 and in 2015 this was extended to include adult sheep. Barber's Pole worm is the most important round worm parasite of sheep and goats in the world and in the Australian summer rainfall zone it is the dominant gastrointestinal nematode genus of small ruminants.

Control and prevention of Haemonchosis is a major priority for producers who rely largely on anthelmintic drugs for this task. Compared to the number available for sheep, few drenches are registered for goats in Australia, mainly because, at 400,000, the number of farmed goats (rangeland goats have been excluded) is much smaller than the population of sheep (73 million in 2011), making caprine registration commercially unattractive.

Goats metabolize most drenches faster than sheep, a situation which can lead to under dosing and the accelerated development of drug resistance, a second factor which deters companies registering anthelmintic products for goats. Furthermore, the safety margin for the organophosphate drugs (which are still effective against *Haemonchus*) is lower for goats than for sheep. Therefore, compared to sheep farmers, goat producers have relatively few options for controlling *Haemonchus* (or other internal parasites).

Barbervax performed well in lambs in 6 field trials conducted in the Northern Tablelands of NSW between 2011 and 2013 (MLA reports B. AHE 0068 and 0214) and during its first season of commercial use over the 2014-15 summer. Furthermore, preliminary overseas trials indicated that the vaccine could also be beneficial for goats. Enquiries made at the Australian Pesticide and Veterinary Medicines Authority (APVMA) indicated that before the vaccine could be sold for use in goats, it would have to be registered; "off label" use was not acceptable. Positive data from a minimum of three efficacy field trials and two safety trials conducted in Australia would be needed to achieve registration.This report mainly describes the outcome of these trials which were done in the Northern Tablelands, although abbreviated data from three overseas studies with goats (not funded by MLA) are also included for comparison and completeness.

MDC co-funding for the NSW trials came from reserve goat levy funds held by the National Residue Survey (NRS) together with co investment made by the Goat Industries Council of Australia (GICA).

2 Projective objectives

2.1 To prove the efficacy of Barbervax against naturally acquired Barber's Pole Worm infection in goats, as evidenced by a reduction in faecal worm egg count.

2.2. To prove the safety of Barbervax in goats by means of two intensively monitored studies.

2.3 Assuming a favourable outcome, to collate the data and submit a registration dossier to the APVMA as an adjunct to the Barbervax dossier for sheep.

3 Methodology

The safety and efficacy trials were subcontracted either to Veterinary Health Research or to the CSIRO McMaster Laboratory, both based in or near Armidale, NSW.

These organisations had previously conducted several successful Barbervax trials in sheep (MLA reports B. AHE 0214, 0068 and 0232).

The designs of the trials in goat kids, which closely followed that used for the lamb studies done during the summer of 2011-12, are presented together with full details of the trial data and statistical methods in their individual reports in Appendix 9.

4 Results

This section is an overview of the safety, efficacy and antibody responses of the kids given Barbervax in the three NSW field trials which were undertaken during the 2013-14 summer. These responses are compared with data obtained previously from goat trials done in Scotland and South Africa.

Full results from each trial are presented in Appendix 9, where they are listed in chronological order.

4.1 Data from three field trials in NSW

4.1.1 Safety

Apart from a mild pyrexia lasting a day or so, no adverse signs were observed after vaccination with Barbervax in any of the goat studies described in Appendix 9, including the two NSW trials reported in 9.3.1 and 9.3.2 where detailed observations were made.

These findings were very simlar to those observed earlier with sheep (see MLA final reports B. AHE 0214, 0068 and 0232).

4.1.2 Efficacy and antibody response

a) Faecal egg counts

As judged by faecal egg counts, the efficacy of Barbervax varied from one premises to another.

The detailed kinetics of the the egg counts in each group is illustrated in the individual trial reports shown in Appendix 9, but the overall efficacy is summarised in Figure 1. At Guyra the counts of the vaccinates were significantly reduced relative to the controls by 73% on average, at Dorrigo the figure was also statistically significant at 44% but at CSIRO, at 17%, it was not statistically signicant.

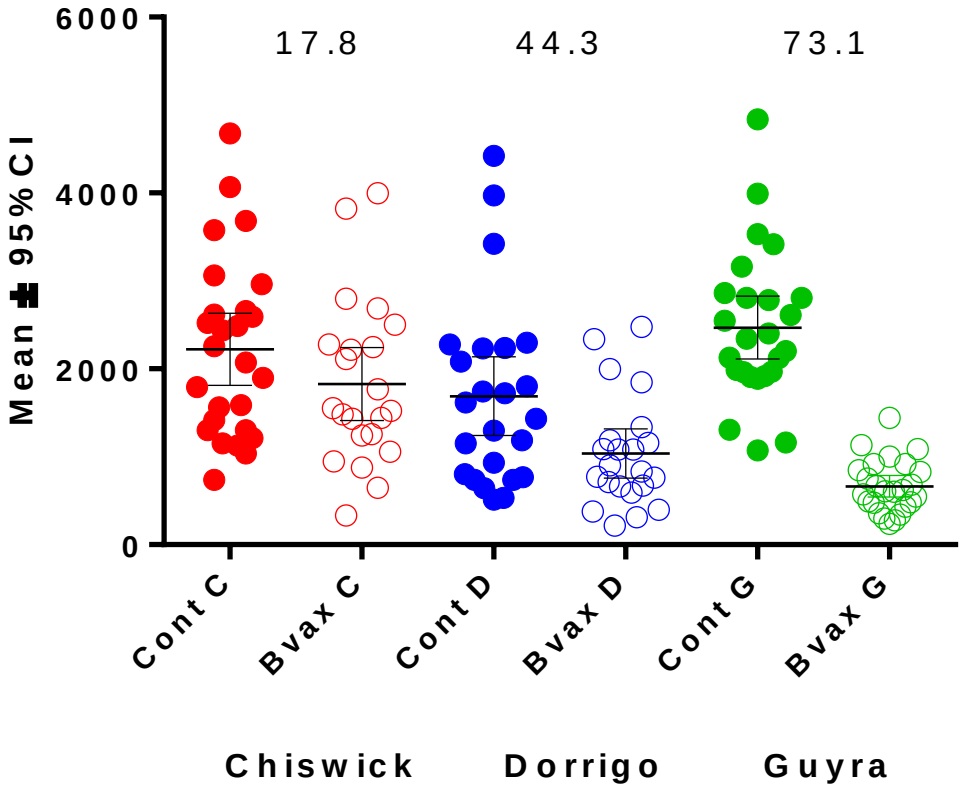


Fig. 1. *Haemonchus* egg counts of goat kids averaged from V3 to end of trial

Individual, group mean and 95% confidence interval egg counts averaged from a week after the third vaccination till the end of the trial. Mean percent protection values calculated for each property are shown at the top of the graph.

b) The anti-vaccine antibody response

The detailed kinetics of this response is illustrated in the individual trial reports shown in Appendix 9. Average and individual titres of the vaccinates in each trial two weeks after the third vaccination (when the response should have reached protective levels) are compared in Figure 2 whereas Figure 3 shows the equivalent data from then until the end of the trial.

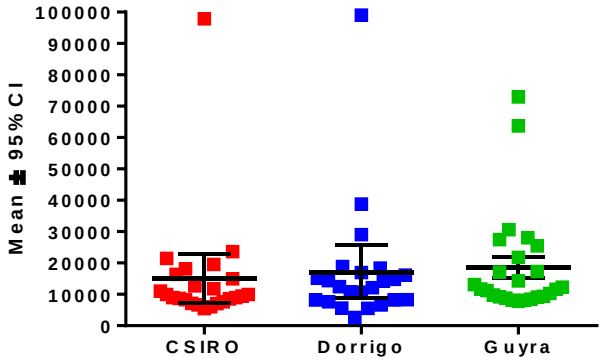


Fig 2 . Anti-vaccine antibody titres of vaccinated goats two weeks after V3

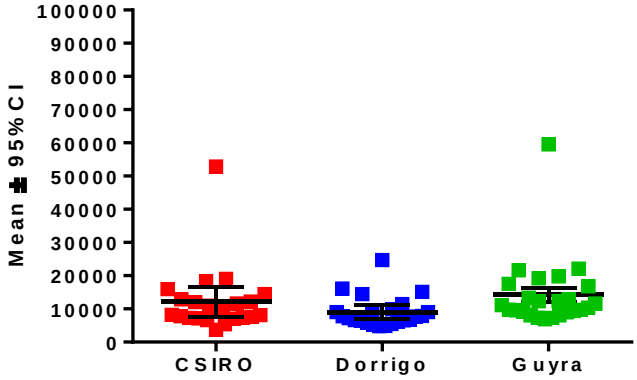


Fig 3 . Anti-vaccine antibody titres of vaccinated goats from two weeks after V3 to trial end

No significant between farm differences were detected between the responses of the vaccinated goats whether measured at the time protective immunity should have developed or averaged across the rest of the trial.

4.2 How did the efficacy and antibody responses of the NSW kids compare with results previously observed in goats overseas?

Data from a preliminary serology-only trial at Moredun indicated that the antibody responses of goats immunised with Barbervax containing small doses of antigen were in the same order as those previously recorded in lambs (Appendix 9.1).

A field trial with 8 month old Boer Goats in South Africa showed that Barbervax could stimulate protective levels of antibodies against a natural challenge infection (Appendix 9.2). This study also indicated that there was little to choose between the responses or efficacies of vaccines containing 5 or 50ug of antigen.

A second South African field trial in Boer goats and Dorper sheep, all grazing the same paddock and all orally infected with the same dose rate of infective larvae, provided some interesting between host species comparisons (Appendix 9.3).

In the unvaccinated control groups egg counts were higher in sheep than goats suggesting sheep were the more susceptible hosts.

Higher antibody titres were stimulated in vaccinated goats than sheep and the vaccine appeared to be more efficacious for goats. The trajectories of the goat and sheep antibody responses were very similar. There was nothing to suggest that the half life of IgG was shorter in goats than sheep.

In summary, both South African trials indicated that Barbervax could be efficacious for Boer goats, results which were contradicted by the CSIRO trial.

5 Discussion

The variation in the degree of vaccine efficacy observed across the three NSW field trials with kids was disappointing. When very similar trials were conducted in NSW lambs over the 2011-12 season all four farms showed consistent efficacy, each at a level roughly equivalent to the Guyra trial and despite a heavy challenge as the Spring was unusually wet (Fig 4).

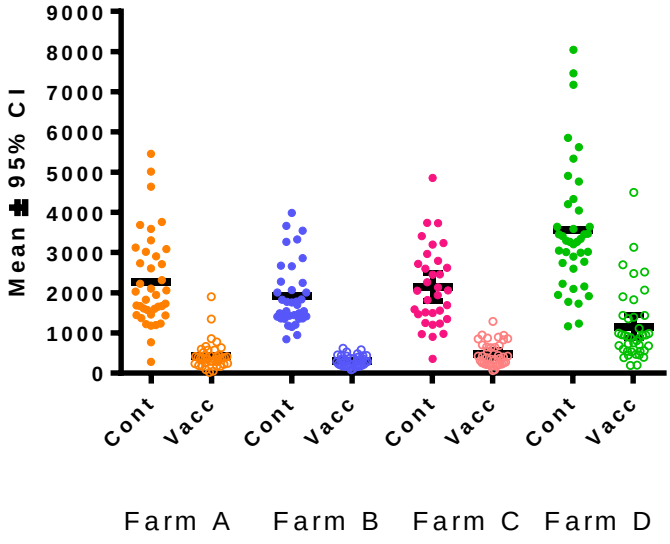


Fig. 4. *Haemonchus* egg counts averaged from V3 to end of trial from vaccinated and control lambs grazing four NSW properties during the 2011-12 summer (see MLA final report 0068)

The reason for the variation in the goat trials is not known as there was little difference in the antibody responses induced by the vaccine on the different properties. The data, albeit limited, from the two S. African trials suggested that Boer goats responded to the vaccine as well as, if not better, than sheep and were better protected. There was no suggestion that the titres stimulated in goats had a shorter half-life, nor did giving a ten fold higher dose of antigen appear to improve the response.

6 Conclusions/recommendations

It was concluded that the efficacy data from the three NSW field trials was too variable for Barbervax to attain APVMA registration for use in kids.

It was not clear why the vaccine failed in one of the trials as the antibody response stimulated was similar to that observed in the other two studies.

Further research might reveal the underlying cause and more field trials could provide more consistent efficacy data, perhaps eventually leading to registration, but this could cost hundreds of thousands of dollars to achieve and with no guarantee of success.

Even if 50% of Australia's 400,000 farmed goats were present in the *Haemonchus* endemic zone, the size of this market would not attract such investment from Wormvax Australia, the Moredun subsidiary which manufactures Barbervax. It will be concentrating on sheep which are at least twenty times more numerous than goats in the region.

It is probable that Barbervax could be a useful tool for a proportion of goat farmers and using Barbervax would make them less reliant on anthelmintic which would preserve the life of these precious drugs.

It is suggested that goat farmers who wished to try Barbervax should use it "off-label", initially in a small portion of their herd and with advice from their vet. Provided they monitor the egg count status of the animals regularily and have access to anthelmintics which are effective on their property for precautionary drenching, they would have little to lose if the vaccine failed except for the cost of the vaccine itself.

7 Key messages

The efficacy of Barbervax in goat kids was too variable to attain registration for that species.

The product would probably be useful on some properties but producers would have to use it "off label".

8 Appendices

8.1 Preliminary Barbervax pilot trial in Scotland

Main Purpose: To determine by serology whether the response to the vaccine in goats was similar to that of sheep

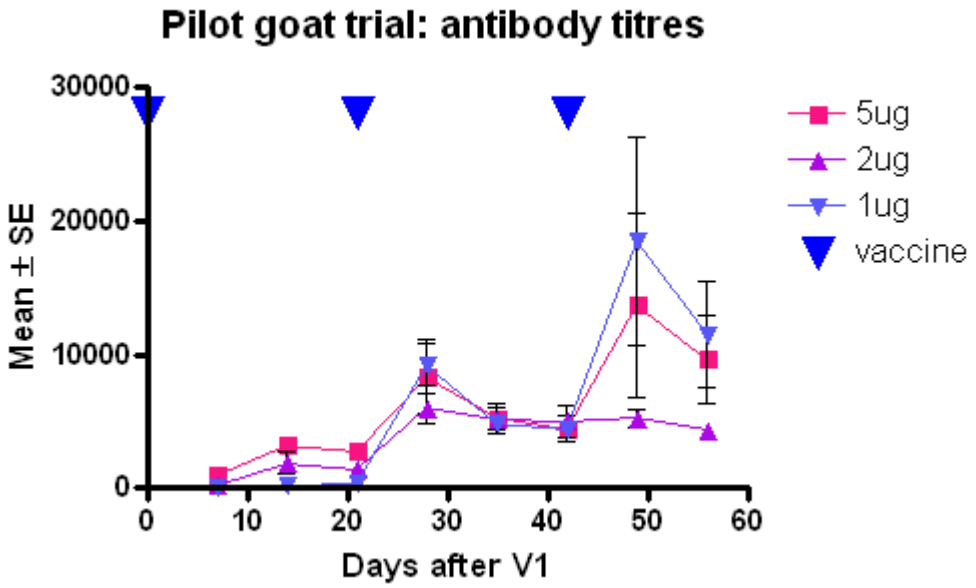
Introduction

The main purpose of this trial was to determine whether the serological response of goats given Barbervax was similar to that of sheep but also to investigate whether the vaccine would induce adverse reactions in goats.

Results

No obvious adverse reactions were observed after Barbervax was administered.

The kinetics of the group mean antibody titres are displayed in the figure below



An increase in the mean anti-vaccine antibody titres was observed in all 3 vaccinated groups by one week after the second vaccination (V2). Titres then declined over the next 2 weeks, but the third and final injection also stimulated a sharp increase in titre, in the 5 and 1ug groups.

Discussion

The results showed that just like lambs, goat kids could mount high titre antibody to the vaccine whether it contained 5, 2 or even 1ug of antigen. The magnitude and kinetics of the response were very similar to those recorded in Moredun pen trials with sheep.

It was concluded that an immunisation regime suitable for Barbervax in lambs was very likely to be appropriate for goat kids too.

8.2 Trials with Barbervax in South Africa

8.2.1 With grazing Boer goats

Introduction: The main purpose of this trial was to determine whether the vaccine would be efficacious for goats which were grazing pasture contaminated with infective *Haemonchus* larvae. Further objectives were to establish a suitable antigen dose and an appropriate immunisation regime. It was anticipated at the outset that some animals might become anaemic during the course of the trial. To prevent potential losses, it was decided that any individual goat with a haematocrit below 15% would be given a precautionary treatment of levamisole and that once 50% or more of the animals in a group had been drenched, then the whole of that group would be treated.

Trial Design Parameters

Start date (first vaccination):	11-Feb-2010
Trial site:	One 0.7ha paddock at Onderstepoort Veterinary Institute, near Pretoria, South Africa.
Breed, age, weight (range) and parasite status of goats	Boer, 8m, 17.8 to 28.4 Kg, drenched on arrival and held on concrete until 9-Mar-2010 when put onto trial paddock.
Groups: number, treatments and size	Two vaccinated groups given either 5 or 50ug antigen with 1mg saponin and a control group immunised with adjuvant alone. Thirteen in each group.
Vaccine: source, formulation, date of manufacture, storage temperature	Moredun antigen batch no 143 made 10 Dec 2009. Formulated to contain 5 or 50 ug antigen and 1mg saponin/ml. Stored 2-8ºC until used.
Volume, route and site of vaccination and immunisation regime	1 ml subcutaneously. Four immunizations given on days, 0, 26, 74, and 112 of the trial..
Challenge infection: dose and source of L3, timing relative to vaccination	Natural *H. contortus* challenge from day 26, when the second vaccination was given
Samples collected	Weekly blood for PCVs and serology and faeces for worm egg counts.

Results

Serology

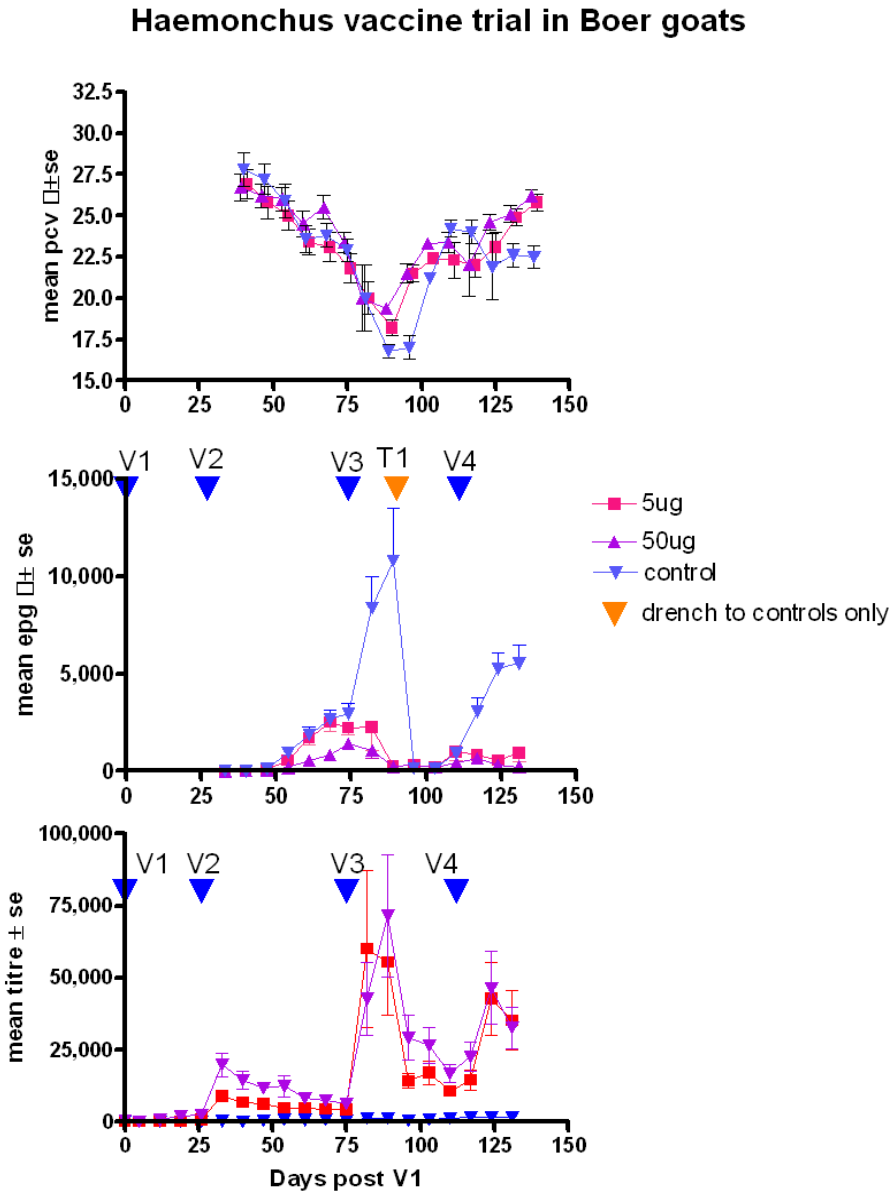
Individual and group mean antibody titres are presented in Fig 1. An increase in the mean anti-vaccine antibody titres was observed in the vaccinated group by one week after the second vaccination (V2). Titres then declined over the next 7 weeks until one week after V3 when they spiked substantially. Mean titres then declined, but the third and final boost (V4)

also stimulated a sharp increase in titre. In contrast control titres remained at background values throughout the trial.

Faecal egg counts

Worm eggs were first detected in most animals after they had been grazing for 3 to 4 weeks (Fig 1). Mean egg counts were quite similar in all three groups after the second vaccination (V2), rising steadily to means aroung 3,000 epg by V3. Control means continued to accelarate peaking at 10,777 epg two weeks later. In contrast, the mean counts of both vaccinated groups fell to only 291 epg at this time (89 days after V1).

By this stage more than half the control goats had required a salvage treatment and so, in accordance with the pre-set plan, all the controls were given levamisole on day 90. Up to this point only two vaccinates had had to be treated.



Mean egg counts in the vaccinated goats remained quite steady for the next two weeks but by 5 weeks after V3 (day 110) had increased to 907 epg. A fourth and final vaccination was given on that date, and the mean counts of the vaccinates subsequently remained below that level for the rest of the trial. In contrast, the controls became re-infected within 4 weeks of their dose of anthelmintic and had mean counts of several thousand epg over the last 3 weeks of the trial, significantly more than the vaccinated groups.

PCVs

The PCVs of all groups were similar on day 33, two to three weeks before the animals started to shed worm eggs. As the egg counts started to rise, the goats in both groups became anaemic with mean PCVs falling close to 20% by Day 75 (Fig 1). One animal in each group had required a precautionary treatment up to this point.

V3 was given to the vaccinated groups on day 75, and by two weeks later the mean PCV of these groups started to rise, a trend which continued after V4 until the end of the trial.

In contrast, 6 further members of the control group needed precautionary treatments on days 82 or 89, triggering a dose of levamisole to the whole of that group on day 90. Thereafter mean control PCVs recovered, but in contrast to those of the vaccinates, declined again during the final two samples of the trial.

Discussion

This natural challenge field trial was the first conducted with Barbervax and was performed before the duration of the immune response to the vaccine in either goats or sheep was known.

There seemed little to choose between 5 or 50 µg as an effective vaccine dose.

It was concluded that repeated administration of the vaccine could substantially reduce anaemia and transmission of the parasite in grazing goats. Had the third immunisation been administered two or three weeks earlier, it is anticipated that the protective effect would have been stronger.

8.2.2 Comparing the response of Boer goat kids and Dorper lambs grazing together.

Introduction

The aim was to confirm the efficacy of Barbervax for goat kids and simultaneously to compare its performance in lambs of a similar age grazing the same pasture. The original intention was to use a natural challenge infection but this failed to materialise and so an artificial challenge was given instead.

Trial design

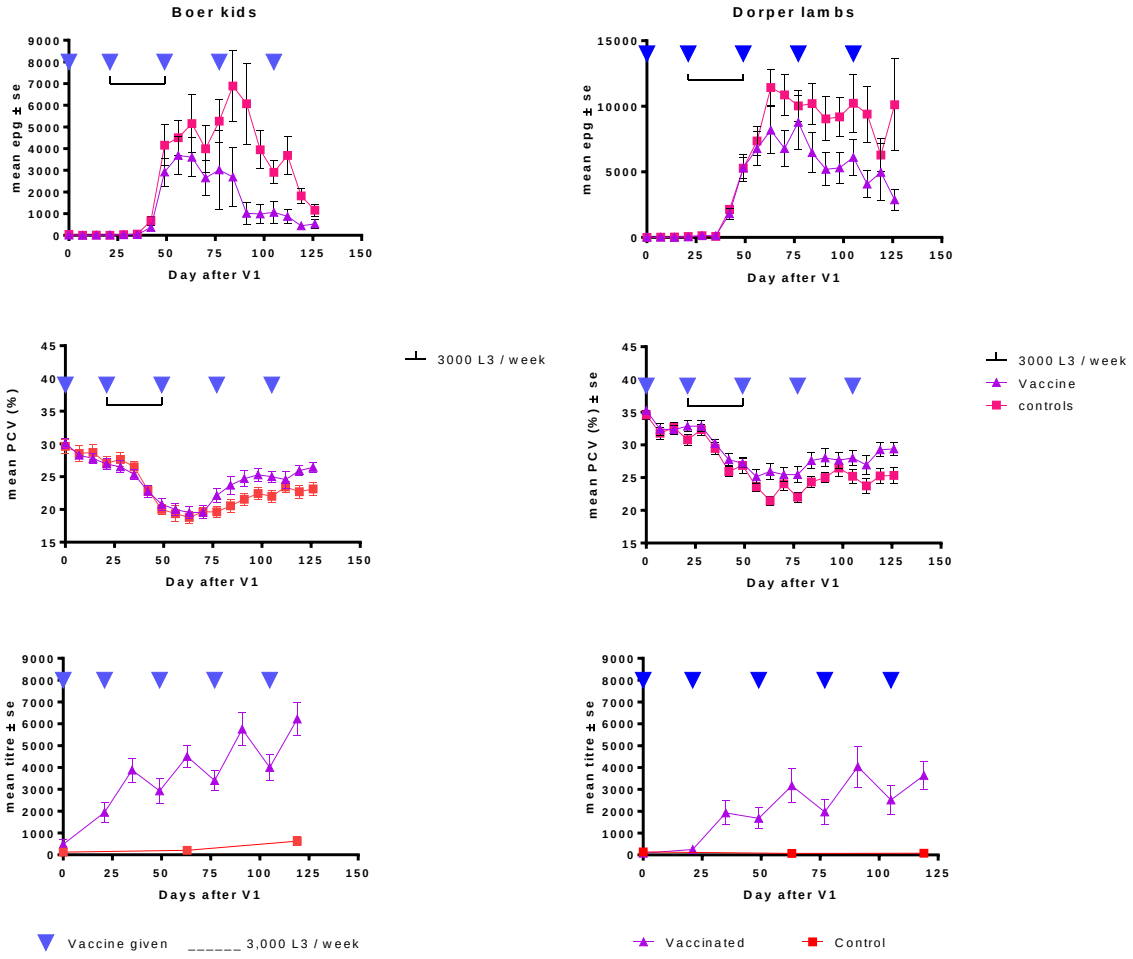
Start date (first vaccination):	24-Jan-2012
Trial site:	Paddock at Onderstepoort Veterinary Institute, near Pretoria, South Africa.
Breed, age, weight (range) and parasite status of sheep and goats	Dorper lambs and Boer goat kids bought in on 3 Nov 2011 when aged 5 months approx, drenched on arrival and held on concrete until 24 Jan when put onto trial paddock.
Groups: number, treatments and size	For each species one vaccinated group given 5 ug antigen with 1mg saponin and a control group immunised with adjuvant alone. Fifteen sheep and 14 goats in each group.
Vaccine: source, formulation, date of manufacture, storage temperature	Moredun antigen batch no 162 made Oct 2011. Formulated to contain 5ug antigen and 1mg saponin/ml. Stored 2-8ºC until used.
Volume, route and site of vaccination and immunisation regime	1 ml subcutaneously. Five immunizations given on days, 0, 21, 49, 67 and 95 of the trial.
Challenge infection: dose and source of L3, timing relative to vaccination	1,500 H. contortus L3 twice a week from 14 Feb for 7 consecutive weeks.
Samples collected	Weekly blood for PCVs and serology and faeces for worm egg counts.

Results

Serology

Vaccination stimulated clear antibody responses in both the kids and the lambs (Fig 1 lowest panels). Mean titres increased as the trial progressed, rising after each boost and declining before the next one. The mean titre in the goats usually exceeded that of the sheep (Fig 2) but the overall shape of their responses was very similar.

Fig 1. Egg counts, PCVs and antibodies in vaccinated and control kids and lambs



Egg counts

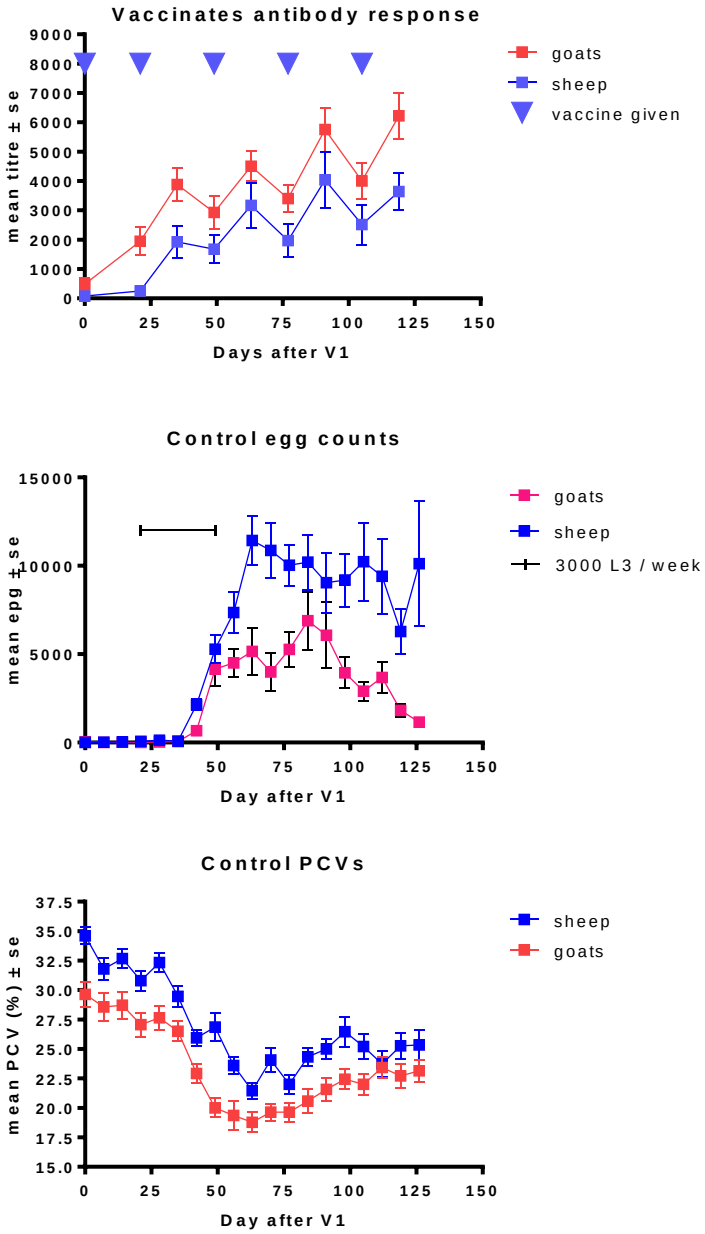
Haemonchus egg counts in the control kids rose from zero to a mean of about 4,000 eggs per g by 4 weeks from the start of the trickle infection (Fig 1, top panel). Their mean count peaked at about 7,000 epg five weeks later before steadily declining to about 1,000 epg by the end of the trial. Initially the mean count of the vaccinated kids followed the same trajectory, but it dropped off more quickly to to reach a mean of about 1,000 five weeks earlier than the controls.

The mean count of the vaccinated kids from a week after the third vaccination (the earliest the vaccine was expected to work) until the end of the trial was significantly lower than control values indicating an efficacy of some 47% (Fig 3).

Mean counts in the control lambs peaked two weeks after the end of the trickle challenge but continued to fluctuate around 10,000 for the rest of the trial (Fig 1). Mean counts In the vaccinated lambs were generally lower, falling from 8,000 to 3000 approximately during the same period, although the overall reduction from V3 (37.1%) was not significantly different (Fig 3).

As judged by faecal egg counts the kids were less susceptible to infection with *Haemonchus* than the lambs which shed significantly ($p<0.01$)more eggs for most of the trial (Fig 3).

Fig 2. Goats vs sheep.

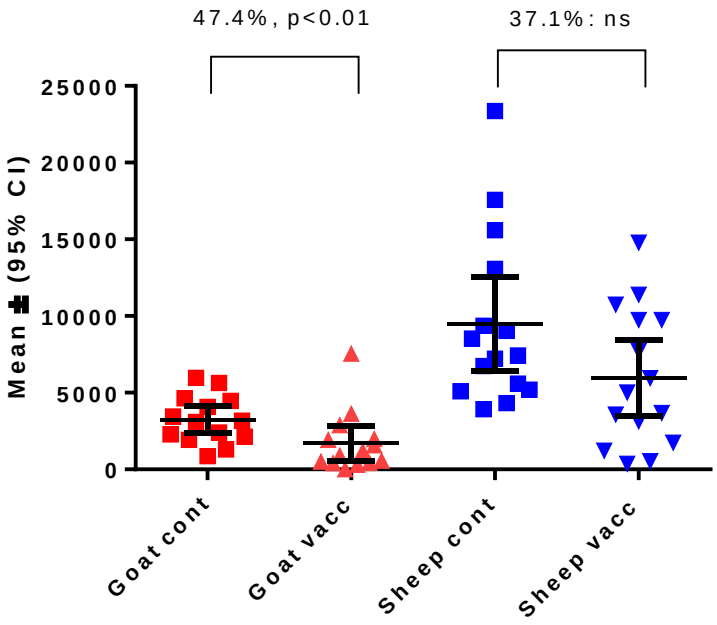


Anaemia

Mean packed red cell volumes (PCVs) dropped steadily during and for a few weeks after the trickle challenge in both groups of goats before recovering by the end of the trial (Fig 1, middle panel). This recovery was more pronounced in the vaccinates than the controls. Between days 56 and 84 seven control and four vaccinated goats required a precautionary drench because their PCVs had fallen to 15% or lower.

Similar trends and differences were observed between the vaccinated and control lambs (Fig 1). However the anaemia was less pronounced in the lambs than in the goats (Fig 2) and no precautionary drenching was required for the members of either lamb group.

Fig 3. Epg from one week after V3



Discussion

The vaccine did reduce *Haemonchus* egg output in both the kids and the lambs (Figs 1 and 3), but the overall degree of protecton was not as strong as seen in subsequent lamb trials in NSW where the challenge was natural (see final reports B. AHE 0214 and B. AHE 0068). Perhaps the challenge dose used in the present trial was excessive.

Whatever the reason, the vaccine stimulated a stronger antibody response in the kids than in the lambs (Fig 2). Presumably that was reflected in their superior overall level of protection (Fig 3).

It was interesting that the lambs had higher egg counts and seemed to be inherently more susceptible to infection with *Haemonchus* than the kids, yet the kids became more anaemic despite an apparently lower worm burden (Fig 2).

Perhaps kids are less resilient to the effects of *Haemonchus* than lambs even though they are less susceptible to infection and acquire immunity more rapidly.

Trickle challenge at 430/day too high?

8.3 Trials with Australian farmed goat kids

8.3.1 Near Dorrigo, NSW

Study Title: A field study to evaluate the safety and efficacy under field use conditions of an *Haemonchus* vaccine when administered subcutaneously to young goats (kids) during times of high parasite challenge.

Study No.: **MIHG2919** **Sponsor Study No.:** NA

Version No.: **3** **Version Date:** Nov 2015

Author: T. Dale

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Investigator:	Name: Quals.: Address:	Timothy Dale B. LISC Veterinary Health Research Pty Ltd Trevenna Road, Armidale, NSW 2350

1. <u>OBJECTIVE</u>

This study was designed to test the safety and efficacy of Barbervax for young goats under field conditions. Barbervax is a *Haemonchus* vaccine registered for use in lambs. Data from this study may be used to support product registration.

2. <u>JUSTIFICATION</u>

Commonly, control of internal parasites in goats has been by drenching with anthelmintics. Haemonchus resistant to many of the commonly used anthelmintics is

widespread in goats across many parts of the world, including Australia. Control by vaccination would reduce dependence on anthelmintics, and hence be of great benefit to both goat and sheep producers, and for the welfare of the animals concerned.

Field use in Australian lambs and trials with goats overseas have shown that Barbervax is effective at reducing host anaemia and parasite egg output. This study aims to determine whether this vaccine is safe and effective in Australian goat kids.

3. 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)

4. TEST SITES

Animal Phase:
"Booma Goats"
1715 Tyringham Road
Dorrigo, NSW, 2453
AUSTRALIA

Laboratory Phase:
Faecal egg counts & larval differentiation
Veterinary Health Research P/L
Colin Blumer Animal Health Laboratory
Trevenna Road
Armidale NSW 2350 Australia
Plasma ELISA anti-vaccine antibodies
Moredun Institute
The Moredun Group
Pentlands Science Park
Bush Loan, Penicuik
Midlothian, Scotland, UK

5. STUDY DATES

Start date (animal phase): 07 OCT 14 (Day -7)
Finish date (safety phase): 21 NOV 14 (Day 38)
Finish date (animal phase): 25 MAR 15 (Day 162)
Finish date (laboratory phase): 15 AUG 15

6. STUDY DESIGN

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

b. Animal Model: This study used kids due to their on-property retention for the full anticipated 12 month withhold period. Study kids grazed normal kidding and weaning paddocks contaminated with *Haemonchus* spp.

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

d. Exclusion and Removal Criteria: All animals in Group 3 were removed at the conclusion of the Safety study on 21 NOV 14 (Day 38).

Animal #23 was excluded from the study on 22 DEC 14 (Day 69) due to animal welfare reasons after it had been attacked by a wild dog.

Animal #31 was excluded from the study on 20 JAN 15 (Day 98). The animal failed to present in the yards and missed the V4 vaccination and could not be found by the grazier.

No further animals were removed or excluded from the study.

e. Allocation: Sixty (60) kids (still suckling their nannies) were randomly selected out of a greater mob, which contained approximately 120 kids, as they presented within the animal handling facility. The age group of the kids ranged from 6 weeks to 1 day old, therefore only kids which matched the animal requirements under 'Section 10. Test System' were picked for the study. Each goat was weighed and given an ear tag with a unique identification number used for allocation purposes.

Animals were then ranked from heaviest to lightest according to bodyweight. The heaviest and lightest animals were allocated to Group 3, the second heaviest to Group 1 and the second lightest to Group 2. The remaining balance, 56 animals were sequentially blocked into 24 blocks each of either 2 or 3 animals as per Table 14 Allocation Table, in Appendix 7. Animals were then blocked into two animals per block or three animals per block and were randomly allocated utilizing the "draw from hat" methodology to Groups 1, 2 or 3. Treatment groups were allocated as such, so that each group had a similar mean live weight distribution.

A further subset of 10 animals was randomly selected from Group 1 and Group 2 to form the comparison animals for the safety component of the study. Each of the animals which were placed within the three animal blocks at allocation was enrolled for the safety study with the tenth animal being randomly selected from the groups by picking a number out of a hat. See Appendix 10, TrialPak Section 11 for allocation data.

Animals were given a second coloured tag with unique ID number for identification purposes over the duration of the study and to identify between the 10 subset animals in Groups 1 and 2 for the safety and efficacy study.

The groups mean bodyweights for both the Safety and Efficacy parts of the study were analysed at allocation for significant differences between groups using One-Way Analysis of Variance and a commercially available software package (Statistix 10.0, 2014). All statistical analysis for both safety and efficacy portions of the study showed that there were no significant differences amongst the groups. See Appendix 10 (TrialPak Section 11) for statistical analysis data.

f. Blinding: Blinding was not undertaken, however, the lab technicians were not told which groups received any treatments to avoid any potential bias.

7. INVESTIGATIONAL VETERINARY PRODUCT

a. Investigational Veterinary Product:

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

b. Source: The vaccine was received via courier from:

WormVax Laboratory
Animal health Laboratory
Department of Agriculture and Food Western Australia
444 Albany Highway
Albany W.A. 6330

c. Storage: IVP was held in Refrigerator 13 between 2 to 8^{o}C with a datalogger recording the temperature inside the fridge for the duration of the vaccine being kept onsite at VHR.

d. Safety: A MSDS was not provided by the Sponsor.

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

f. Drug Disposal: IVP is to be held on premises for a period of up to 12 months before disposal via high temperature incineration.

8. TREATMENT

Animals in Group 1 were retained as untreated controls, but individual animals in Groups 1, 2 or 3 were treated with a short acting anthelmintic if any of the following criteria were reached:

- ***H.contortus*:** individual egg count >10,000 epg or if the blood haemoglobin concentration < 7.5 g/100mL
- **Other genera:** (indicated by larval differentiation): the individual egg count > 1500 epg, or scouring was evident. For a flock treatment, the upper limit was a mean of 1000 epg.
- **Scouring**: Individuals were treated if above an AWI Scour Score of 3.

Group 2 animals were vaccinated on five occasions over the duration of the study, initially 4 then 6 weeks apart with a single dose of the IVP.

Group 3 animals were vaccinated on two occasions 4 weeks apart with two doses of the IVP.

a. Dose Calculation: Dose volume was 1.0 mL of IVP by one subcutaneous injection (single dose) or 2.0 mL by subcutaneous injection (double dose) given as two separate 1mL injections at two different injection sites a minimum of 5 cm apart.

b. Dose Preparation: The IVP was already prepared and ready for use. The IVP was transported on ice bricks and gently shaken for 10 seconds prior to the 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	Objective	Number of Animals	Treatment	Treatment Days
1	Efficacy	25	Unvaccinated control	Not applicable
2	Efficacy	25	Single vaccinated	Approx. Day 0, 28, 56, 98 and 140
1a	Safety	10 (subset of Group 1)	Unvaccinated control	Not applicable
2a	Safety	10 (Subset of Group 2)	Single vaccinated	Day 0 (V_1), and Day 28 (V_2)
3	Safety	10	Double vaccinated	Day 0 (V_1) and Day 28 (V_2)

The IVP was administered using a NJ Phillips Vaccine Gun with 18G x ½ inch needles subcutaneously beneath the skin on the left side of the neck. Animals given two doses of vaccine were injected at two separate sites approximately 5 cm apart on the left hand side of the neck. Animals were observed immediately post-treatment for any signs abnormal signs. No abnormal clinical signs were observed by VHR personnel or the grazier over the duration of the study.

Animals enrolled in the Safety study were monitored for rectal temperature, behavior, abnormal clinical signs, and skin thickness and swelling at the injection site one day prior to vaccination, on the day of vaccination and thence Days 1, 2, 3, 6, 8 and 10 post each V1 and V2 vaccination. No abnormalities clinical signs, swellings or lumps were observed at any stage over the duration of the safety study.

9. SCHEDULE OF EVENTS

Table 2: Schedule of Events

Study Day	Date	Event
Pre-Study		Obtained Animal Ethics approval received IVP and located a commercial goat farm out at Dorrigo with suitable *Haemonchus* spp. infection.
-7	07.10.2014	Sixty (60) of the largest and heaviest kids (visually assessed) were weighed and tagged. Animals were allocated to groups based upon bodyweight in descending order. Statistical analysis was conducted to verify that all groups were homogeneous. Animals were given a second tag which was coloured and had a unique ID which was used for the remainder of the study. Blood and faecal samples were collected from all animals. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. Groups 1 and 2 blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
-1	13.10.2014	Safety Study: Conducted clinical observations and recorded temperatures from all kids enrolled in the safety study (see **Deviation #2**). No abnormalities were detected. Animal #200 was weighed and enrolled into the study (see **Deviation #1**).
0	14.10.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. All kids had faecal samples collected and received a short acting anthelmintic drench of ZOLVIX. Animals in Groups 2 and 3 were treated with IVP (V1) vaccine. Blood samples were collected from Group 2. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. The blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.

Study Day	Date	Event
1	15.10.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected and no lumps, excessive heat or swelling present at injection site. Animals #25 and #44 were drenched, vaccinated, faecal samples collected and blood sample collected (See **Deviation #3**)
2	16.10.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected and no lumps, excessive heat or swelling present at injection site.
3	17.10.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected and no lumps, excessive heat or swelling present at injection site.
6	20.10.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected.
8	22.10.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected.
10	24.10.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected.
27	10.11.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected.
28	11.11.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected. All animals had faecal samples and blood collected. A haemoglobin analysis was conducted on all animals, none required a drench. All kids in Groups 2 & 3 were treated with IVP (V2) vaccine. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. The blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
29	12.11.2014	Conducted clinical observations and collected temperatures from all kids. A clinical examination was conducted on Animal #17 due to a low body temperature.
30	13.11.2014	Conducted clinical observations and collected temperatures from all kids. A follow-up clinical examination was conducted on animal #17 with no abnormalities detected, return to normal health.

Study Day	Date	Event
31	14.11.2014	Conducted clinical observations and collected temperatures from all kids. No abnormalities detected.
34	17.11.2014	Conducted clinical observations and collected temperatures from all kids. Animals #50 & #59 appeared to be losing condition, all other animals no abnormalities detected
36	19.11.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. Clinical examination was conducted on animals #50 and #59 based upon the comments from the previous visit. All other animals, no abnormalities detected.
38	21.11.2014	Conducted clinical observations and collected temperatures from all kids. No abnormalities detected.
40	23.11.2014	Grazier weaned kids as per normal management practice.
55	08.12.2014	Day 42 and all the events which were to occur, did not (See **Deviation #4**)
59	12.12.2014	Collected blood, faecal samples and conducted haemoglobin analysis on all animals. Animals in Group 2 received 1mL of (V3) vaccine. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. The blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
69	22.12.2014	All trial animals were weighted, collected blood, faecal samples and conducted haemoglobin analysis on all animals. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. The blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
84	06.01.2015	Collected blood, faecal samples and conducted haemoglobin analysis on all animals. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. The blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.

Study Day	Date	Event
98	20.01.2015	Collected blood, faecal samples and conducted haemoglobin analysis on all animals. Animals in Group 2 received 1mL of (V4) vaccine. All trial animals were given a double dose of Abamectin drench. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. The blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
106	28.01.2015	Drenched six animals received a salvage drench of Zolvix. Faecal samples were collected all trial kids enrolled in the study.
112	03.02.2015	Collected blood, faecal samples and conducted haemoglobin analysis on all animals. Three animals received a salvage drench. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. The blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
126	17.02.2015	Collected blood, faecal samples and conducted haemoglobin analysis on all animals. Four animals received a salvage drench. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. The blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
140	03.03.2015	Collected blood, faecal samples and conducted haemoglobin analysis on all animals. Animals in Group 2 received 1mL of (V5) vaccine. Two animals received a salvage drench. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. The blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.

Study Day	Date	Event
154	17.03.2015	Collected blood, faecal samples and conducted haemoglobin analysis on all animals. Eight animals received a precautionary drench. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. The blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
162	25.03.2015	Study Terminated (See **Amendment #1**)
	15.06.2015	Dispatched plasma samples to Moredun.

10. TEST SYSTEM

Species:	Goats	**Number:**	60
Breed:	Boer and Boer Cross	**Source:**	Booma Goats
Weight:	8.5 – 26.0 kg (at induction)	**Health & special requirements:**	Healthy unweaned animals. No treatment with long acting anthelmintics.
Sex:	Males and Females		
Age:	3 – 8 weeks of age		
Method of ID:	Unique ID tag for allocation and Unique coloured ear tag with number for group ID		

11. ANIMAL MANAGEMENT

a. Animal Welfare: Study animals were managed similarly and with due regard for their welfare. Animals were observed twice weekly for health problems according to AEC requirements. Animals were handled in compliance with UNE AEC no. 14-058 approved 01AUG14, and any applicable local regulations.

b. Health Management: (Concurrent Medications, precautionary Drenches and Deaths) Study animals were clinically observed at each sampling time-point. A precautionary drench was administrated to the animals when the individual animal's haemoglobin levels fell below 7.5 g/100mL or had a high scour worm burden. A summary is provided below:

Day -7: Animals #2, #5, #21, #32, #34, #36, #41, #42 and #57 all received a salvage drench with 6mL of CYDECTIN ORAL (1 g/L Moxidectin, Batch: 1304903, Expiry: August 2015) due to animals having scours.

Day -6: Animal #41 found dead by grazier. Animal died possibly due to heavy worm burden as scouring was evident (see Deviation #1).

Day 0: All animals enrolled in the trial received 5 mL of ZOLVIX (25 g/L Monepantel, Batch: 806305, Expiry: January 2016) as per Section 9 Schedule of Events in the protocol.

Day 1: Animals #25, #39 and #44 were all drenched to their bodyweight with ZOLVIX (25 g/L Monepantel, Batch: 806305, Expiry: January 2016) as they were missing on Day 0

due to miss-muster and drenching these animals brought them into line with the other kids.

Day 48: Animal #47 was found dead in the paddock by the grazier. Animal was suspected to have died due to worms as the animal had scours. No autopsy was performed as the grazier did not find the animal dead until approx. 12 hours post death.

Day 59: Animals #23 and #49 received a salvage drench of 2 mL ZOLVIX (25 g/L Monepantel, Batch: 806305, Expiry: January 2016). Animal #23 had a low haemoglobin level whilst animal #49 had scours.

Day 69: Animals #10 and #11 received a salvage drench of 2 mL ZOLVIX (25 g/L Monepantel, Batch: 806305, Expiry: January 2016) due to animals having scours.

Day 76: Grazier gave Animal #11 a salvage drench of 2 mL ZOLVIX (25 g/L Monepantel, Batch: 806305, Expiry: January 2016) due to animal having scours.

Day 84: Animals #21, #35 and #38 received a salvage drench to their bodyweight with ZOLVIX (25 g/L Monepantel, Batch: 806305, Expiry: January 2016) due to low haemoglobin level. Animals #41 and #43 were found dead in the paddock by grazier. No autopsy was performed as only the skeletons of the carcass remained.

Day 98: All animals were given a narrow spectrum salvage drench of VIRBAMEC ORAL (0.8 g/L Abamectin, Batch: 50754V1, Expiry: March 2016) to reduce the trichostrongylus spp. population within the goats.

Day 105: Animal #1 was treated by the Grazier with 4 mL of ZOLVIX (25 g/L Monepantel, Batch: 806305, Expiry: January 2016) and 2 mL of Tylan 200 (200 mg/mL Tylosin) due to animals having scours.

Day 106: Animals #12, #31, #35, #37, #42, #44 and #45 received a salvage drench of ZOLVIX (25 g/L Monepantel, Batch: 806305, Expiry: January 2016) due to low haemoglobin level on Day 98.

Day 112: Animals #12, #17 and #46 received a salvage drench of STARTECT (10 mg/mL Derquantel and 1 mg/mL Abamectin) due to low haemoglobin level.

Day 126: Animals #2, #5, #6 and #21 received a salvage drench of STARTECT (10 mg/mL Derquantel and 1 mg/mL Abamectin) due to low haemoglobin level.

Day 140: Animals #11 and #35 received a salvage drench of STARTECT (10 mg/mL Derquantel and 1 mg/mL Abamectin) due to low haemoglobin level.

Day 154: All animals received 6mL of VIRBAMEC ORAL (0.8 g/L Abamectin, Batch: 50754V1, Expiry: March 2016). Animals #1, #2, #12, #15, #19, #32, #37 and #50 received an additional salvage drench of ZOLVIX (25 g/L Monepantel, Batch: 806305, Expiry: January 2016) due to low haemoglobin count. Animals #7, #16 and #36 were all found dead in the paddock by the grazier. Kids were suspected to have been attacked by wild dogs as grazier had lost several kids previously to dogs and were actively trying to trap and removed the animals. No autopsy was performed as there was very little to nothing left of the carcasses.

Day 168: Grazier drenched all animals with ZOLVIX (25 g/L Monepantel) to bring animals back in line with their commercial herd.

c. Housing: Routine management practices were followed. Study animals in Groups 1, 2 and 3 were all grazed together in normal kidding prepared paddocks and weaning paddocks contaminated by *Haemonchus* spp. Goats were grazed upon native and improved pastures, primarily containing kikuyu grass, with *ab lib* access to water from paddock dam or stock trough (see **Note to File #2**).

d. Animal Disposal: Any study animal treated with the IVP (Groups 2 and 3) are not to enter the human food chain for 12 months past the last treatment with the IVP (V5 Group 2, V2 Group 3.

12. STUDY PROCEDURES

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

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

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

d. Sample Storage, Transfer & Disposal: Sample storage, transfer and disposal were recorded. Replicate 1 plasma samples were dispatched for analysis on ice-bricks via same day 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.

13. ASSESSMENT OF EFFECTS

a. Body Weights: Animals were weighed according to VHR SOP FLD-406 at intervals outlined in section 9 - Schedule of Events. Individual animal weights were recorded. Animal weigh scales were checked pre- and post-weighing with calibrated test weights according to VHR SOP FLD-406 and recorded on a "Scale Verification" record. Body weights and body weight change over the duration of the study was compared between groups to determine treatment effects (if any).

b. Clinical observations: Clinical observations were performed according to VHR SOP FLD-409 at intervals outlined in section 9 – Schedule of Events. Clinical observations will be recorded on a "Clinical Observations Record" form. VHR study personnel in charge of goats monitoring will particularly be concerned about reactions at injection site, skin reactions, swelling, body temperature, abnormal behavior and any abnormal clinical signs.

c. Clinical Examinations: Any animal showing abnormal clinical signs on observation as per 13b above were clinically examined according to VHR SOP FLD-409 and recorded on a "Clinical Examination Record". Clinical examinations were performed according to VHR SOP FLD-409 at intervals outlined in section 9 - Schedule of Events. Key objective and subjective clinical examination parameters were compared between groups to determine treatment effects (if any). Appropriate samples for biochemistry or hematology were not required to be taken and no further treatment was required.

d. Rectal Temperatures: Rectal temperatures (Body temperatures) were recorded according to VHR SOP FLD-419 at intervals outlined in section 9 - Schedule of Events. Rectal temperatures during the study were compared between groups to determine treatment effects (if any). Any animal showing serious abnormal body temperatures were clinically examined.

e. Blood analysis: Single blood samples were collected from each animal using 18 gauge x ½ inch needles into 8 mL lithium heparin gel separator vacutainers according to VHR SOP FLD-414 at intervals outlined in section 9 – Schedule of Events. Haemoglobin levels in the blood were analysed using the HEMOCUE Hb ANALYSER. Blood samples were processed for collection of replicate 1 and 2 plasma samples on the day of collection, or following overnight refrigeration, according to VHR SOP PRO-506. Samples were individually labeled with the study no., animal no., study date & day, sample type and replicate. Samples were stored frozen (-10 to -30ºC) in temperature monitored freezers pending dispatch for analysis. Replicate 1 plasma samples were forwarded frozen to Moredun Institute laboratories for ELISA anti-vaccine antibody analysis at the completion of the animal phase of the study.

f. Faecal Egg Counts/larval Differentiation: Faecal samples were collected and recorded according to VHR SOP-416 at intervals outlined in section 9 – Schedule of Events. Faecal samples will be individually labeled with the animal ID. Faecal egg counts were performed according to VHR SOP PRO-510 and larval differentiation according to VHR SOP PRO-512. Faecal egg counts and larval differentiation were compared by group to determine treatment effects (if any).

14. STATISTICAL ANALYSIS

Data from body temperature and bodyweight were entered into a computer spreadsheet (Microsoft EXCEL); validated and group arithmetic means calculated using the spreadsheet.
Parasite burdens for each animal will be determined from faecal egg counts. Percentage efficacy will be calculated according to:
100 x (1 - Group Mean (treated)/Group Mean (untreated))

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

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

One-Way Analysis of Variance, its equivalent non-parametric test and additional statistical analysis was performed as appropriate by the Sponsor's professional statisticians. See Appendix 5 ANOVA statistics for body temperature and Appendix 6 for ELISA test.

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. Protocol Amendments & Deviations:

Amendment #1: Study Terminated on the 25 MAR 15 (Day 162) after communication between VHR and Sponsor. The IVP was not having the anticipated response or desired effect. IVP had constant challenge due to heavy worm burden and severe drench resistance in host animal. There was minimal difference between the vaccinated and

control groups therefore it was decided to terminate the study early. Enough data was gathered over the duration of the study that results should suffice.

Deviation #1: Animal #102 died, suspected from worms on 08 OCT 14, one day after being enrolled into the study. At the time of enrolment, the kid received a drench of Cydectin (6 mL) because the animal had scours and showed signs of a worm infection. Therefore a new kid #200 was enrolled into the study. As treatment had not occurred, a new goat #200 was enrolled into the study to replace animal #102. Kid #200 had the same weight (9.5 kg) which allowed the animal to be substituted into the trial without any change to the allocation or statistics. Animal #200 is a part of both the safety and efficacy study. This deviation had no impact upon the study.

Deviation #2: Ten (10) animals were not clinically observed and did not have their temperatures recorded on Day -1. The Grazier miss-mustered the paddock as some nannies and kids had moved into the next paddock. This deviation should have minimal impact upon the study as the same events occurred the following day.

Deviation #3: All the activities which were to occur to animals #25 and #44 on Day 0 (14 OCT 14) eg. drench, vaccination, temps, FEC, bled and clinically observed, occurred on Day 1 (15 OCT 14). On Day 0, the animals had jumped the fence. The goats were found in the next paddock over, the following day. This deviation should have minimal impact on the study as kids were brought back-in-line with all other study animals.

Deviation #4: Day 42 and all events which were to occur on this day did not. Due to an oversight and both goat studies having activities on the one day, Day 42 samplings at the Dorrigo test site were not collected. As a result there is no data between the V2 and V3 vaccinations for this site. This deviation had minimal effect upon the study; even though the data for Day 42 is missing, no key event e.g. vaccination was missed and enough data was collected over the duration of the study.

Deviation #5: Animals #23 and #31 were removed from the study. The damage sustained to #23 from the attack left the animal in a debilitated state. The animal was treated with antibiotics which may have had an effect upon the vaccine. The grazier humanly put down the kid two days later due to animal welfare concerns.

Animal #31 was excluded from the study because it missed the V4 vaccination on Day 98 due to miss mustering. The grazier could not find the animal the following day to give it the vaccine and therefore could not be brought back into line with the remaining animals in Group 2.

b. Notes to File:

Note to File #1: For identification purposes, all kids enrolled into the study were given two flock tags.

1. White flock tag with unique ID
2. Coloured flock tag with unique ID

The white ear tag numbers were written on by hand using a permanent marker and were used for allocation purposes whilst in the field on Day -7. A coloured flock tag which relates to groups was used to identify animals involved in both the safety and efficacy

studies. A summary of the tag numbers and ID's can be found below in Table 3. All data obtained from kids enrolled in the study from Day -1 onwards were identified using 'Tag No.' from 1 – 60.

Table 3. Summary of Tag Colours and Numbers

Unique ID	Tag No.	Colour	Unique ID	Tag No.	Colour
101	16	White	131	58	Green
102	41	Pink	132	44	Pink
103	42	Pink	133	20	White
104	1	Blue	134	32	Red
105	2	Blue	135	45	Pink
106	51	Green	136	46	Pink
107	52	Green	137	33	Red
108	53	Green	138	11	Blue
109	17	White	139	21	White
110	3	Blue	140	47	Pink
111	18	White	141	34	Red
112	26	Red	142	22	White
113	4	Blue	143	35	Red
114	5	Blue	144	48	Pink
115	6	Blue	145	36	Red
116	54	Green	146	12	Blue
117	7	Blue	147	59	Green
118	55	Green	148	13	Blue
119	27	Red	149	37	Red
120	28	Red	150	23	White
121	43	Pink	151	14	Blue
122	19	White	152	38	Red
123	56	Green	153	39	Red
124	8	Blue	154	49	Pink
125	29	Red	155	24	White
126	57	Green	156	40	Red
127	9	Blue	157	60	Green
128	10	Blue	158	25	White
129	30	Red	159	50	Pink
130	31	Red	160	15	Blue

Note to File #2: On the 23rd November 2014 (Study Day 40) the grazier weaned all the trial animals as per their normal management practice.

Over the following 30 days the kids enrolled in the efficacy study were being run in a larger mob of up to approximately 900 other goats (including Billie's, Nannies and kids).

On 06 January 2015 (Study Day 84), Investigator asked grazier if all remaining animals could be held in a separate paddock by themselves. Grazier had setup a small paddock with an electric fence to hold the trial animals.

Note to File #3: Weighed and recorded the bodyweight of all kids enrolled in the study on Day 28 at grazier's request. The bodyweights recorded on Day 28 were used to calculate the amount of drench required when giving the kids a salvage drench due to high epg or low haemoglobin count.

c. Change of Study Personnel: There was no change of study personnel over the duration of the study.

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

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

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

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

h. Study Report: The original signed Study Report with raw data, Analytical Report(s) and Statistical Report (*delete as appropriate*) 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

PART 1: SAFETY

a. Clinical observations: No reactions at the injection site, swelling or skin reactions were observed over the duration of the study. Several animals were observed to have had scours/ diarrhoea over the duration of the safety study and two enrolled animals were clinically examined.

b. Clinical examinations: Any animal showing abnormal signs during observation were clinically examined. On Day 29, Animal #17 had a very low rectal temperature. The temperature was taken 3 times with the same result. A follow up exam was conducted the following day which the kid's temperature had risen back to within the normal range. On Day 36, Animals #59 and #50 had elevated heart rate and were shivering as a result of getting wet in the rain.

c. Rectal Temperatures: These were measured in all the animals enrolled in the safety study. Group mean and standard error rectal temperatures are plotted in Fig 1.

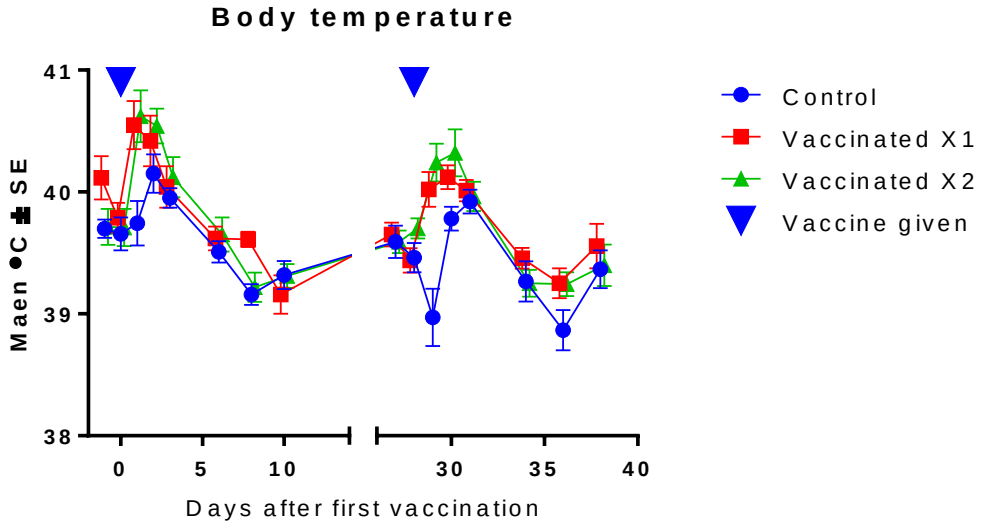

Fig 1. Group mean and standard error rectal temperatures. (Note that for reasons of clarity the data plotted in Fig 1 has been slightly offset along the X-axis).

Rectal temperature measurements suggested a transient rise of less than one degree centigrade a day after each immunization (Fig 1) in both vaccinated groups compared to the controls. Analysis revealed that this difference was only statistically different for Group1, the animals which received the single dose of vaccine (See Appendix 5 for ANOVA results).

PART 2: EFFICACY

a. Deaths

Seven animals died during the course of the trial. In two cases, these deaths were ascribed to scouring, in 3 to wild dogs and in 2 the cause was unknown (see Section 11b above for details).

b. Faecal Egg counts

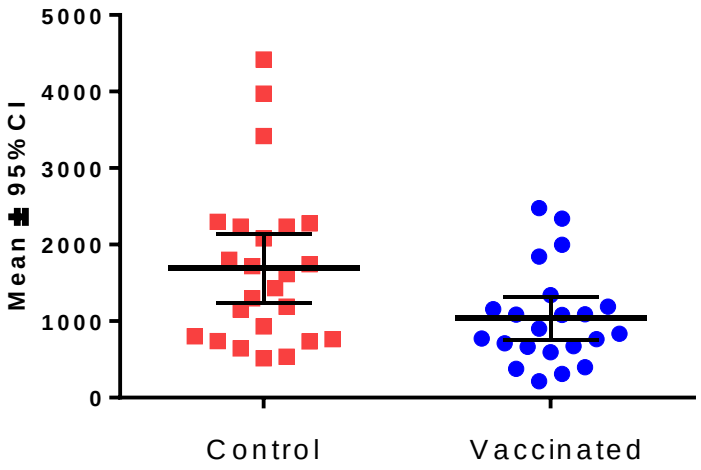

Fig 2. Overall effect of Barbervax on *Haemonchus* egg counts from Day 69 (the earliest Barbervax could have an effect) until the end of the trial.

Barbervax significantly (P<0.01 by t-test) reduced mean *Haemonchus* egg counts over the course of the trial (Fig 2). On most of the sampling days after the third vaccination control *Haemonchus* counts were higher than vaccinates (Table 4, Fig 3).

Table 4. Group mean *Haemonchus* egg counts and efficacy on each sample day.

	Sample Day	Control	Vaccinated	%Efficacy
	-7	113	373	
V1	**0**	363	283	
V2	**28**	57	59	
V3	**59**	165	86	
	69	178	131	26.0
	84	1016	675	33.5
	98	2068	2897	-40.1
V4	**106**	1750	1359	22.3
	112	1483	639	56.9
V5	**126**	1534	1318	14.1
	140	4236	1231	70.9
	154	1611	708	56.1

(The earliest the vaccine could have an effect was from Day 98)

The lowest panel in Fig 3 shows the presence of large numbers of scour worm eggs in both groups. These reached a mean of about 1000 epg by the end of December, were removed by the Abamectin treatment in late January but by March had resumed their original high

levels. The *Haemonchus* egg count of the control kids was relatively unaffected by the dose of abamectin given to all kids, but the difference between mean vaccinate and control *Haemonchus* eggs, seemed to improve after this treatmant had been given.

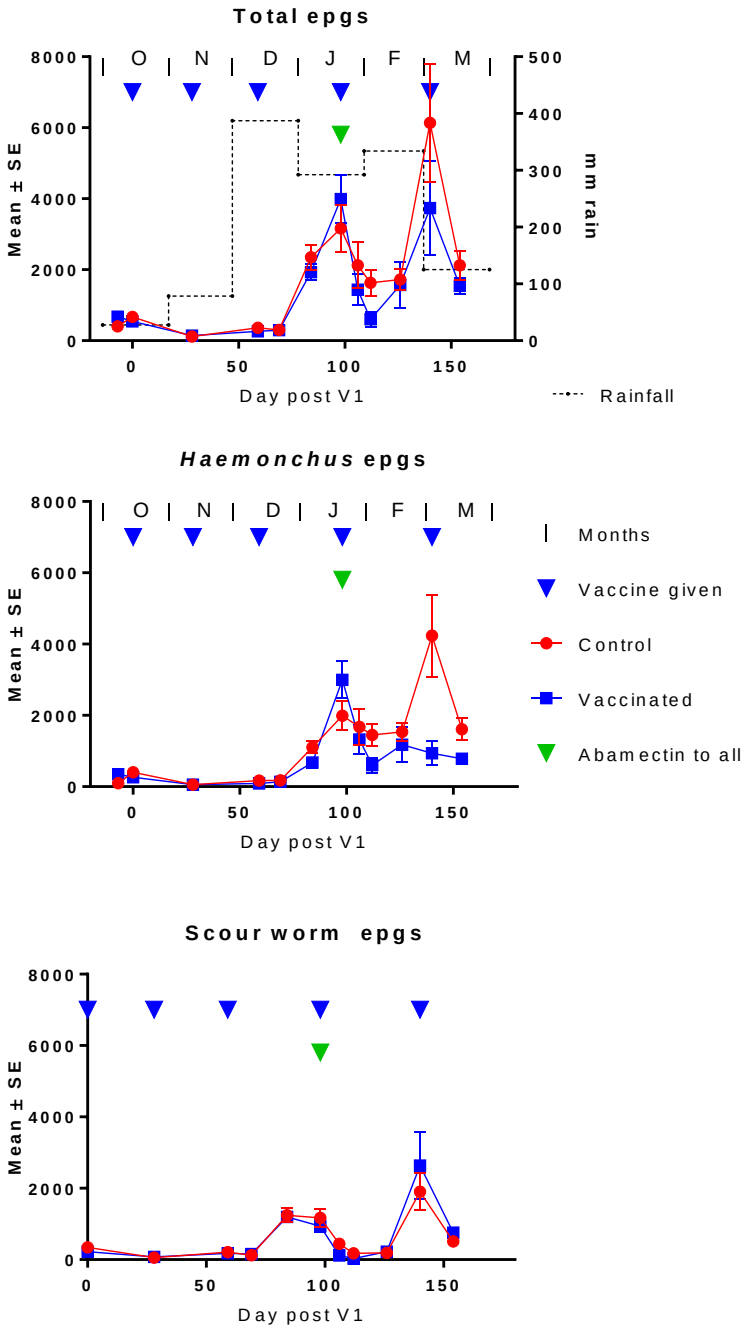

Fig 3. Kinetics of interventions, group mean total, *Haemonchus* and scour worm egg counts

c. Blood haemoglobin concentrations:

Means of the two groups of kids are depicted in the central panel of Fig 4. Up until late January group mean blood haemoglobin concentrations were very similar, but higher

values were recorded in the vaccinates after this time, reflecting the difference in mean Haemonchus egg counts recorded during February and March (Fig 4, top panel).

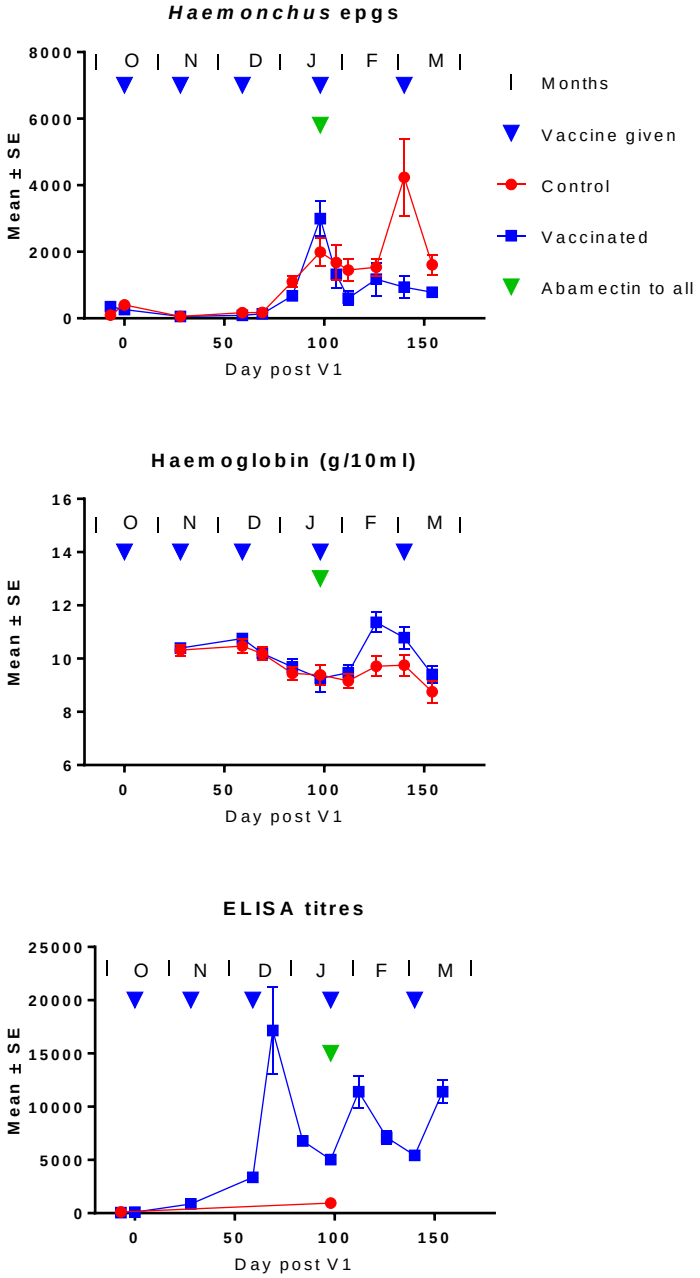

Fig 4. Kinetics of *Haemonchus* egg counts in relation to blood haemoglobin concentrations and anti-vaccine antibody titres.

d. Antibody concentrations in the vaccinates rose gradually after the second vaccination but much more sharply after the third, to peak at a mean of more than 15,000 in December (Fig 4 lower panel). Further spikes in titre were observed in the vaccinates after the fourth and fifth injections, but mean titres were always maintained above 5,000.

e. Body Weights: During the study the animals gained on average 6.4 kg indicating they had maintained a normal growth pattern. Group bodyweights are summarized in Table 4. There were no other significant differences in group mean bodyweights during the study.

Table 5: Summary of Group Mean Bodyweights

Mean Weight (kg) (+/-SD)			
Group	**Day -7**	**Day 28**	**Day 69**
1	13.8[a] (3.1)	17.5[a] (3.5)	19.8[a] (3.6)
2	13.4[a] (2.9)	17.4[a] (3.6)	19.8[a] (3.4)

[ab] Means in the same row with the same subscript are not significantly different at p≤0.05

18. CONCLUDING REMARKS

SAFETY

It was concluded that vaccination caused 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.

None of the abnormal clinical signs that were occasionally observed seemed to be connected with vaccination.

The overall conclusion was that the adverse signs associated with administration of Barbervax were mild and commercially acceptable. The data essentially confirmed that previously observed in sheep and broadly agreed with published descriptions of the side effects of other ruminant vaccines containing saponin.

EFFICACY

Whilst Barbervax did significantly reduce *Haemonchus* egg counts of the vaccinated kids over the duration of the trial, the protective effect was not as strong as that observed in previous field trials with kids or lambs, being confined to the latter part of the study during February and March. This was despite an apparently normal antibody response to the vaccine being stimulated by three injections from mid December onwards.

More than 300mm rain fell each month at Dorrigo during December, January and February, (Fig 3 top panel) which, when coupled with summer temperatures, would have made for excellent conditions for nematode parasites to develop on the pasture.

Despite this, *Haemonchus* specific egg counts in the control kids were unexceptional, peaking during early March at roughly 4,000 eggs per g (Fig 3, middle panel). It was a different story for scour worms (*Trichostrongylus* and *Teladorsagia* spp. in roughly equal proportions (Table 10 in Appendix 3) where mean egg counts of both groups exceeded 1100

per g in December and January and 2,000 per g in early March, indicating the continued presence of these genera in pathogenic numbers for the duration of the trial.

A faecal egg count reduction test had indicated the presence of macrocyclic lactone resistant *Haemonchus* on the property and, since *Trichostrongylus* resistant to this anthelmintic are rare, an attempt was made to selectively remove them from both groups by means of a double dose of abamectin. This was successful in that the scour worm egg count of both groups was almost abolished for four weeks afterwards whereas *Haemonchus* egg counts in the non-vaccinated animals were relatively unaffected (Fig 3, middle and lower panels). Interestingly, the efficacy of Barbervax seemed to improve after this treatment (Fig 3, middle panel). However, if the presence of scour worms was adversely affecting the protective capability of Barbervax, this was not reflected in the antibody titres the vaccine generated.

Detailed comparison of the present results with those from other similar field trials with goat kids and lambs will be made and presented in the final report of this project.

ABBREVIATIONS

VICH	International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Products
GCP	Good Clinical Practice
APVMA	Australian Pesticides and Veterinary Medicines Authority
MORAG	Veterinary Manual of Requirements and Guidelines
WAAVP	World Association for the Advancement of Veterinary Parasitology
VHR	Veterinary Health Research
SOP	Standard Operating Procedure
WHP	Withholding Period
MSDS	Material Safety Data Sheet
ID	Identification
UNE	University of New England
AEC	Animal Ethics Committee
QA	Quality Assurance
kg	Kilograms
g	Grams
µg	Micrograms
mg	Milligrams
mL	Millilitres
IVP	Investigational Veterinary Product
^{o}C	Degrees Celsius
epg	Eggs Per Gram
AWI	Australian Wool Innovation
cm	Centimeters
FEC	Faecal Egg Count
Hb	Haemoglobin
No.	Number
BPM	Beats Per Minute

8.3.2 Near Guyra, NSW

Study Title: A field study to evaluate the safety and efficacy under field use conditions of an *Haemonchus* vaccine when administered subcutaneously to young goats (kids) during times of high parasite challenge.

Study No.: **MIHG3067** **Sponsor Study No.:** N/A
Version No.: **3** **Version Date:** Nov 2015
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
Sponsor Monitor & Rep.:	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

19. OBJECTIVE

This study aimed to confirm the efficacy and the safety under field use conditions of a *Haemonchus* vaccine when administered subcutaneously to young goats (kids) during times of high parasite challenge. Data from this study may be used to support product registration.

20. JUSTIFICATION

Commonly, the treatment of internal parasites in goats 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 goats across many parts of the world including Australia. The increasing prevalence of anthelmintic resistant strains of nematode parasites of grazing animals has led to greater emphasis on finding new means of combating this problem. The use of a vaccine to control these parasites would reduce dependence on

anthelmintics, and hence are of great benefit to both goat and sheep producers, and for the welfare of the animals concerned.

Initial field trials in Australian sheep and overseas trials with goats have shown that the vaccine in question is effective at reducing host anaemia and parasite egg output. This study aims to confirm:

1. Efficacy when young goats (kids) are vaccinated pre-weaning thence approximately 4 - 6 weekly for the duration of the *Haemonchus* season according to 'label directions' (single dose – efficacy component).
2. Safety when young goats (kids) are vaccinated on two occasions - pre-weaning thence approximately 4 weeks later according to 'label directions' (single dose) and double 'label directions' (double dose).

21. 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)

22. TEST SITE(S)

Animal Phase:

"Urandangie"
2224 Wongwibinda Road
Wollomombi, NSW, 2356
AUSTRALIA

Laboratory Phase:

Faecal egg counts & larval differentiation
Veterinary Health Research P/L
Colin Blumer Animal Health Laboratory
Trevenna Road
Armidale NSW 2350 Australia

Plasma ELISA anti-vaccine antibodies
Moredun Institute
The Moredun Group
Pentlands Science Park
Bush Loan, Penicuik
Midlothian, Scotland, UK

23. STUDY DATES

Start date (animal phase): 25 November 2014 (Day -7)

Finish date (safety phase): 09 January 2015 (Day 38)

Finish date (efficacy phase): 01 June 2015 (Day 181)

Finish date (laboratory phase): 17 Aug 2015

24. STUDY DESIGN

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

b. **Animal Model:** This study used kids due to their on-property retention for the full anticipated 12 month withhold period. Study kids were grazed upon normal kidding

prepared paddocks and weaning paddocks which were contaminated with *Haemonchus* spp.

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

d. **Exclusion and Removal Criteria:** All animals in Group 3 study were removed from the study after Day 38 (09 JAN 15) at the conclusion of the Safety study. No further animals were removed or excluded from the study up till the end of the animal phase

e. **Allocation:** Fifty nine (59) (see **Deviation #2**) kids still suckling nannies were selected as they presented within the animal handling facility. The mob of the kids ranged from 3 to 6 weeks of age which matched the animal requirements. Each kid was weighed and given an ear tag with a unique identification number which was used for allocation purposes.

f. Animals were then ranked from heaviest to lightest according to their bodyweight. The heaviest animal was allocated to Group 3, the second heaviest to Group 1 and the lightest to Group 2. The remaining balance of (56) animals were sequentially blocked into 24 blocks each of either 2 or 3 animals as per Table 14, Appendix 5. Animals within two animal blocks or three animal blocks were randomly allocated utilizing the “draw from hat” methodology to Groups 1, 2 or 3. Treatment groups were allocated as such, so that each group had a similar mean live weight distribution.

g. A further subset of 10 animals, were randomly identified from Group 1 and Group 2 to form the comparison animals for the safety component of the study. Each of the animals which were placed within the three animal blocks at allocation was enrolled for the safety study with a 10^{th} animal being randomly selected from the groups by picking a number out of a hat. See Appendix 8 (TrialPak Section 11) for allocation data.

h. Animals were given a second coloured tag with unique ID number for identification purposes over the duration of the study and to identify between the 10 subset animals in Groups 1 and 2 for the safety and efficacy study.

i. The groups mean bodyweights for both the Safety and Efficacy parts of the study were analysed at allocation for significant differences between groups using One-Way Analysis of Variance and a commercially available software package (Statistix 10.0, 2014). All statistical analysis for both safety and efficacy portions of the study showed that there were no significant differences amongst the groups. See Appendix 8 (TrialPak Section 11) for statistical analysis data.

j. **Blinding:** Blinding was not undertaken, however, the lab technicians were not told which groups received any treatments to avoid any potential bias.

25. INVESTIGATIONAL VETERINARY PRODUCT

a. Investigational Veterinary Product (IVP):

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

b. **Source:** The vaccine was received via courier from:
WormVax Laboratory
Animal health Laboratory
Department of Agriculture and Food Western Australia
444 Albany Highway
Albany W.A. 6330

c. **Storage:** IVP was held in Refrigerator 13 between 2 to 8^{o}C with a datalogger recording the temperature inside the fridge for the duration of the vaccine being kept onsite at VHR.

d. **Safety:** A MSDS was not provided by the Sponsor.

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

f. **Drug Disposal:** IVP is to be held on premise for a period of up to 12 months before disposal via high temperature incineration.

26. TREATMENT

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

- ***H.contortus*:** the egg count was over 10,000 epg or if the blood haemoglobin concentration fell below 7.5 g/100mL.
- **Other genera:** (indicated by larval differentiation): the individual animal egg count raised over 1500 epg, or scouring was evident. For a flock treatment, the upper limit was a mean of 1000 epg.
- **Scouring**: Individuals were treated if above an AWI Scour Score of 3.

Group 2 animals were vaccinated on five occasions over the duration of the study, initially 4 thence 6 weeks apart with a single dose of the IVP.

Group 3 animals were vaccinated on two occasions 4 weeks apart with two doses of the IVP.

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

Dose Preparation: The IVP was already prepared and ready for use. IVP was transported on ice bricks and gently shaken for approximately 10 seconds prior to the first treatment.

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

Table 1: Treatment Regime

Group	Target	Number of Animals	Treatment	Treatment Days
1	Efficacy	25	Unvaccinated control	Not applicable
2	Efficacy	25	Single vaccinated	Days 0, 28, 56, 98 and 140
1a	Safety	10 (subset of Group 1)	Unvaccinated control	Not applicable
2a	Safety	10 (Subset of Group 2)	Single vaccinated	Day 0 (V_1) and Day 28 (V_2)
3	Safety	10	Double vaccination	Day 0 (V_1) and Day 28 (V_2)

The IVP was administered using an NJ Phillips Vaccine Gun with 18G x ½ inch needles, subcutaneously beneath the skin on the left side of the neck. Animals that required two doses of vaccine were treated in two separate sites approximately 5 cm apart on the left hand side of the neck. Animals were observed immediately post-treatment for any signs abnormal signs. No abnormal clinical signs were observed over the duration of the study by VHR personnel or the grazier.

Animals enrolled in the Safety study were monitored for rectal temperature, behavior, abnormal clinical signs, skin thickness at injection site and swelling at injection site. Monitoring started one day prior to vaccination, on the day of vaccination and thence Days 1, 2, 3, 6, 8 and 10 post each V1 and V2 vaccination. No abnormalities clinical signs, swellings or lumps were observed in any animal at any stage of the safety study.

27. SCHEDULE OF EVENTS

Table 2: Schedule of Events

Study Day	Date	Event
Pre-Study		Obtained Animal Ethics approval, received IVP and located a commercial goat farm at Guyra with suitable *Haemonchus* spp. infection
	03.11. 2014	Visited test site to induct animals and start the trial. Animals were to light and trial was delayed (see **Deviation #1**).
-7	25.11.2014	Fifty nine (59) of the largest and heaviest kids (visually assessed) were weighed and tagged. Animals were allocated into groups based upon bodyweight in descending order. Statistical analysis was conducted to verify that all groups were homogeneous. Animals were given a second tag which was coloured and had a unique ID which was used for the remainder of the study. Blood and faecal samples were collected from all animals. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups (see **Deviation #3**). Group 1 and 2 blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
-1	01.12. 2014	Safety Study: Conducted clinical observations and recorded temperatures from all kids enrolled in the safety study. No abnormalities were detected.
0	02.12.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected. All kids had faecal samples collected and received a short acting anthelmintic drench. Animals in Groups 2 and 3 were treated with IVP (V1) vaccine. Blood samples were collected from Group 2. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. Group 2 blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
1	03.12.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected.
2	04.12.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected.
3	05.12.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected.
6	08.12.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected.
8	10.12.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected.
10	12.12.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected.
27	29.12.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected.

Study Day	Date	Event
28	30.12.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected. All animals had faecal samples and blood collected. A haemoglobin analysis was conducted on all animals, none required a drench. All kids in Groups 2 and 3 were treated with IVP (V2) vaccine. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. Group 2 blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
29	31.12.2014	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities detected.
30	01.01.2015	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities detected.
31	02.01.2015	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities detected.
34	05.01.2015	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected.
36	07.01.2015	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected.
38	09.01.2015	Safety Study: Conducted clinical observations and collected temperatures from all kids. No abnormalities were detected.
42	09.01.2015	Collected FEC and blood samples from all animals. Conducted a haemoglobin analysis on all animals. No kid required a salvage drench. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. Group 2 blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
56	27.01.2015	Collected FEC and blood samples from all animals. Conducted a haemoglobin analysis on all animals. Animal #31 was given a salvage drench. All kids in Group 2 received 1mL of (V3) vaccine. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. Group 2 blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
70	10.02.2015	All kids were weighed. Collected FEC and blood samples from all animals. Conducted a haemoglobin analysis on all animals. No kid required a salvage drench. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. Group 2 blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
84	24.02.2015	Collected FEC and blood samples from all animals. Conducted a haemoglobin analysis on all animals. No kid required a salvage drench. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. Group 2 blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.

Study Day	Date	Event
98	10.032015	Collected FEC and blood samples from all animals. Conducted a haemoglobin analysis on all animals. All kids in Group 2 received 1mL of (V4) vaccine. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. Groups 1 and 2 blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
112	24.03.2015	Collected FEC and blood samples from all animals. Conducted a haemoglobin analysis on all animals. Animals #7 and #44 required a salvage drench. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. Group 2 blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
126	07.04.2015	Collected FEC and blood samples from all animals. Conducted a haemoglobin analysis on all animals. Animal #28 required a salvage drench. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. Group 2 blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
140	21.04.2015	Collected FEC and blood samples from all animals. Conducted a haemoglobin analysis on all animals. All kids in Group 2 received 1mL of (V5) vaccine (See **NTF #1**). Animal #23 required a salvage drench. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. Group 2 blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
154	05.05.2015	Collected FEC and blood samples from all animals. Conducted a haemoglobin analysis on all animals. Animals #1, #21, #22, #25 and #48 all required a salvage drench. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. Group 2 blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
164	15.05.2015	Grazier added 2 Billie's into trial mob (see **NTF #2**).
168	19.05.2015	Collected FEC and blood samples from all animals. Conducted a haemoglobin analysis on all animals. Animals #10 and #20 required a salvage drench. Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. Group 2 blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
181	01.06.2015	Weighed all animals. Collected FEC and blood samples from all animals. Conducted a haemoglobin analysis on all animals. Drenched all animals enrolled in study (at grazier's request). Upon return to VHR, faecal samples were given to lab for FEC and coproculture by Groups. Groups 1 and 2 blood samples were processed via high speed centrifuge, plasma harvested and stored frozen in two replicas in separate -20^{o}C freezers.
	15.06.2015	Dispatched plasma samples to Moredun.

28. TEST SYSTEM

Species:	Goats	**Number:**	60
Breed:	Boer and Boer Cross	**Source:**	Commercial goat farm
Weight:	7.2 – 16.4 kg (at induction Day -7)	**Health & special requirements:**	Healthy unweaned animals. No treatment with long acting anthelmintics.
Sex:	Male (both castrates and intact) and Females		
Age:	3 to 6 weeks		
Method of ID:	2 unique ID tags (one for allocation and one coloured group tag)		

29. ANIMAL MANAGEMENT

a. **Animal Welfare:** Study animals were managed similarly and with due regard for their welfare. Animals were observed twice weekly by the grazier for health problems according to AEC requirements. Animals were handled in compliance with UNE AEC no. 14-058 approved 01 AUG 14, and any applicable local regulations.

b. **Health Management:** (Concurrent Medications and Salvage Drenches)
Day 0: All animals received 2mL of Zolvix (Batch: 806305, Expiry: JAN 2016) as per Section 9. Schedule of Events in the Protocol.
Day 56: Animal #31 received 3mL of Zolvix (Batch: 806305, Expiry: JAN 2016) due to low blood haemoglobin <7.5g/100mL.
Day 112: Animals #7 and #44 received 3mL of Zolvix (Batch: 806305, Expiry: JAN 2016) due to low blood haemoglobin <7.5g/100mL.
Day 126: Animal #28 received 4mL of Zolvix (Batch: 806305, Expiry: JAN 2016) due to low blood haemoglobin <7.5g/100mL.
Day 140: Animal #23 received 3mL of Zolvix (Batch: 806305, Expiry: JAN 2016) due to low blood haemoglobin <7.5g/100mL.
Day 154: Animals #1, #21, #22, #25 and #48 all received 4mL of Zolvix (Batch: 806305, Expiry: JAN 2016) due to low blood haemoglobin <7.5g/100mL.
Day 168: Animals #10 and #20 received 3mL of Zolvix (Batch: 806305, Expiry: JAN 2016). Animal #10 due to low blood haemoglobin <7.5g/100mL on the day and animal #20 had a high FEC count from the previous timepoint.
Day 181: All animals received 5mL of Zolvix (Batch: 807113, Expiry: DEC 2016) at grazier's request.

c. **Housing:** Routine management practices were followed. Routine management practices were followed. Study animals in Groups 1, 2 and 3 were all graze together in normal kidding prepared paddocks and weaning paddocks contaminated by *Haemonchus* spp. Goats were grazed upon native and improved pastures with *ab lib* access to water from paddock dam.

d. **Animal Disposal:** Any study animal treated with the IVP (Groups 2 and 3) are not to enter the human food chain for 12 months past the last treatment with the IVP (V5 Group 2, V2 Group 3). An "Animal Accountability" form was completed.

30. 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 on day -7 at the start of the study and prior to administration of treatment.

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

d. Sample Storage, Transfer & Disposal: Sample storage, transfer and disposal were recorded. Replicate 1 plasma samples were dispatched for analysis on ice-bricks via same day 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

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. **Clinical Observations:** Clinical observations were performed according to VHR SOP FLD-409 at intervals outlined in section 9 – Schedule of Events. Clinical observations will be recorded on a "Clinical Observations Record" form. VHR study personnel in charge of goats monitoring will particularly be concerned about reactions at injection site, skin reactions, swelling, body temperature, abnormal behavior and any abnormal clinical signs.

c. **Clinical Examinations:** Clinical examinations were recorded at intervals outlined in section 9 - Schedule of Events. Digital still images were recorded as appropriate. Key objective and subjective clinical examination parameters were compared between groups to determine treatment effects, if any, and are detailed in the results section of the Study Report.

d. **Rectal Temperatures:** 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.)

e. **Blood Analysis:** Duplicate blood samples were collected from each animal using 18 gauge needles into 8 mL LH Lithium Heparin Sep 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. Frozen plasma samples were forwarded to Moredun Institute laboratories for haematology and biochemistry analysis on 15 June 15. 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. 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. STATISTICAL ANALYSIS

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

Parasite burdens for each animal were estimated from faecal egg counts. Percentage efficacy was calculated according to:

Efficacy = 100 x (1 - Group Mean (treated) / Group Mean (untreated))

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

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

One-Way Analysis of Variance, its equivalent non-parametric test and / or additional statistical analysis was 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

a. **Protocol Amendments & Deviations:**

Deviation #1: The study was to start on the 03 NOV 14 (Day -7). However, due to some of the kids only being 3 days of age and underweight, the trial is to be postponed for 3 weeks. At allocation on Day -7 (03 NOV 14) approximately 15 animals were weighed and bodyweights recorded, nearly all of the animals were too small. The stress of bleeding, faecal sample collection and the grazier marking them all in a single day was thought to be too much and could possibly result in some of the animals dying. On an animal welfare basis the trial was delayed for 3 weeks. Starting date became 25 NOV 41 (Day -7). This deviation had a positive impact as the goats being older will allow them to tolerate the handling and events better causing them less discomfort.

Deviation #2: Only 59 animals were enrolled in the study instead of the required 60. During the allocation, only 9 animals were enrolled in Group 3 for the safety study. In the week prior to Day -7 (25 NOV 14) feral predators were seen in the paddock where the nannies and kids were being held. On the day of induction, only 59 kids were present in the facilities with the assumption (by the grazier) that foxes killed the other kids. At allocation, it was decided that only 9 animals would be in Group 3 for the safety study as the efficacy part of the study would run for a total of 182 days and therefore Groups 1 & 2 should have 50 animals for a full dataset. This deviation had no impact upon the study as groups still had an adequate sample size.

Deviation #3: Due to the size of the kids, only small quantities of faeces were recovered for the lab to perform faecal egg counts and coprocultures by groups. On Day -7, only enough faecal samples were collected for the lab to be able to perform a FEC count and no cultures were made due to lack of sample. On Day 0 only enough sample was collected for either a FEC or coproculture, lab staff were directed to conduct coprocultures only. As FEC was conducted on Day -7, it was deemed that the samples from Day 0 were to be used for a coproculture only to gain data on the worm larvae present (if any) within the kids. As all the animals were drenched on Day 0 (as per protocol), therefore it was deemed a good idea to try and recover larvae over the faecal egg counts because the information would be more beneficial. This deviation had no impact upon the study.

b. Notes to File:

Note to File #1: Animal #30 (Group 2) received 2 x 1.0mL of Barbervax (V5) vaccine on Day 140, 21 April 2015.

The kid had jumped a fence from the vaccinated kids, back into the unvaccinated animals without the trial personnel's knowledge.

Note to File #2: Grazier added two Billie's into the trial mod on 15 May 15 (Day 164) as part of his normal management practice. Goats were still run in a single mob under the same conditions.

c. **Change of Study Personnel:** There was no change of study personnel over the duration of the study.
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.

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

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

f. **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.

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

35. RESULTS

PART 1: SAFETY

a. **Clinical observations:**
The results of individual goats are recorded in Table 6 in Appendix 3.

b. **Body temperatures:**
Group mean and standard error rectal temperatures are plotted in Fig 1 and individual daily values presented the Tables 7a and 7b in Appendix 3.

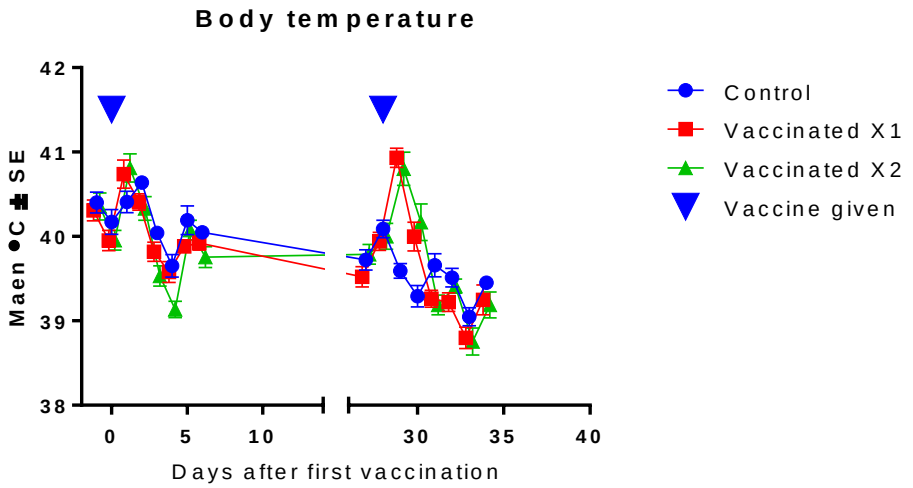

Fig 1. Group mean and standard error rectal temperatures. (Note that for reasons of clarity the data plotted in Fig 1 has been slightly offset along the X-axis).

Rectal temperature measurements suggested a transient rise of up to about one degree centigrade a day after each immunization (Fig 1) in both vaccinated groups compared to the controls. Giving two injections on the same day did not affect the outcome.

PART 2: EFFICACY

a. Deaths

No animals died during the course of the trial.

b. Faecal Egg counts

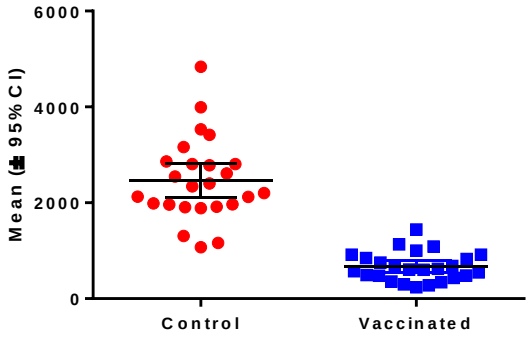

Fig 2. Overall effect of Barbervax on *Haemonchus* egg counts between February and May.

Barbervax substantially reduced the *Haemonchus* egg counts over the course of the trial (Fig 2). On most of the sampling days control Haemonchus counts were significantly higher than vaccinates (Table 3, Fig 3), though it was interesting to note that the difference between the groups was lowest at the time V4 and V5 were due but was then restored to higher levels by two weeks after each of these boosts.

Table 3. Group mean *Haemonchus* egg counts and efficacy on each sample day.

	Sample Day	Control	Vaccinated	%Efficacy
	-7	0	0	
V1	0			
V2	28	0	0	
	42	6	4	
V3	56	310	400	-28.9
	70	608	165	72.9
	84	1536	521	66.1
V4	98	1608	1521	5.4
	112	2367	294	87.6
	126	3645	900	75.3
V5	140	2016	1709	15.3
	154	6087	56	99.1
	168	2610	429	83.6
	181	1825	506	72.3

(the earliest the vaccine could have an effect was from Day 70)

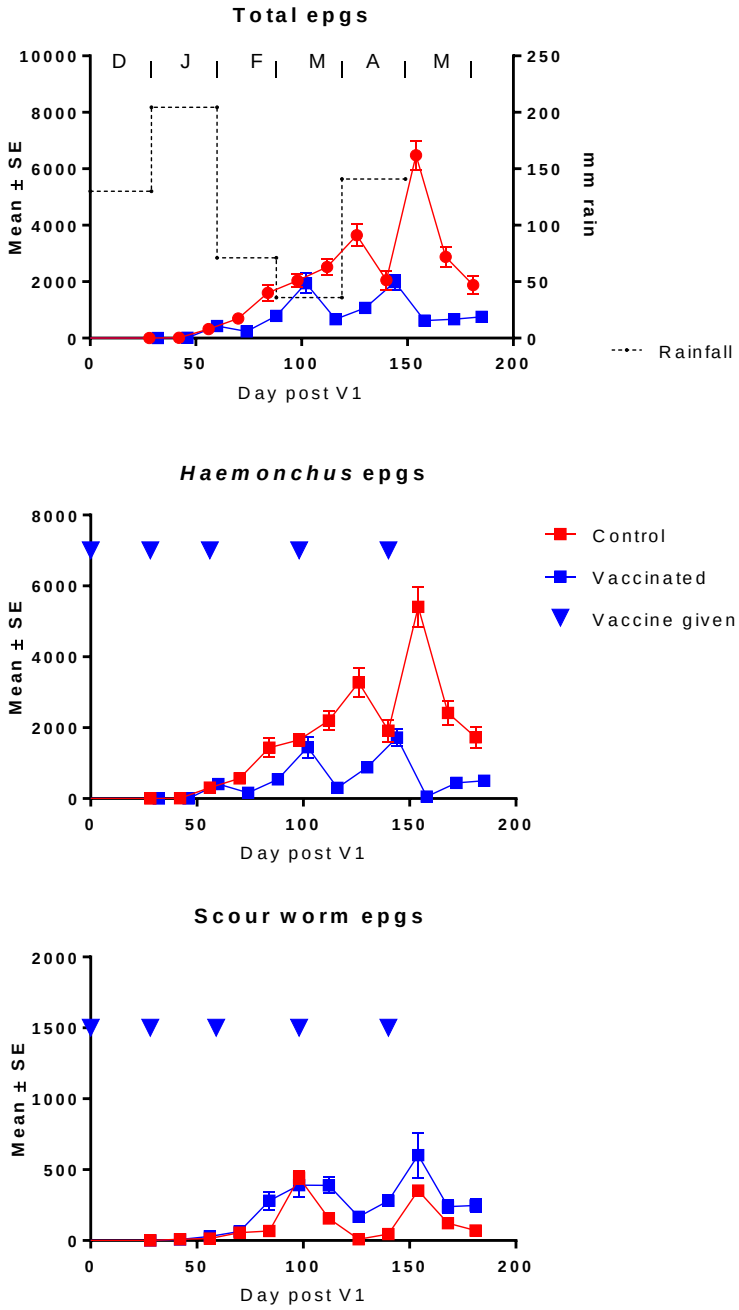

Fig 3. Kinetics of interventions, group mean total, *Haemonchus* and scour worm egg counts.

As expected the nematode eggs were predominantly *Haemonchus,* though a few hundred eggs per gram from scour worms were also detected in both groups at most samplings from Day 100 onwards (Fig 3 and Table 9 in Appendix 3). The scour worms were *Trichostrongylus* and *Teladorsagia* genera.

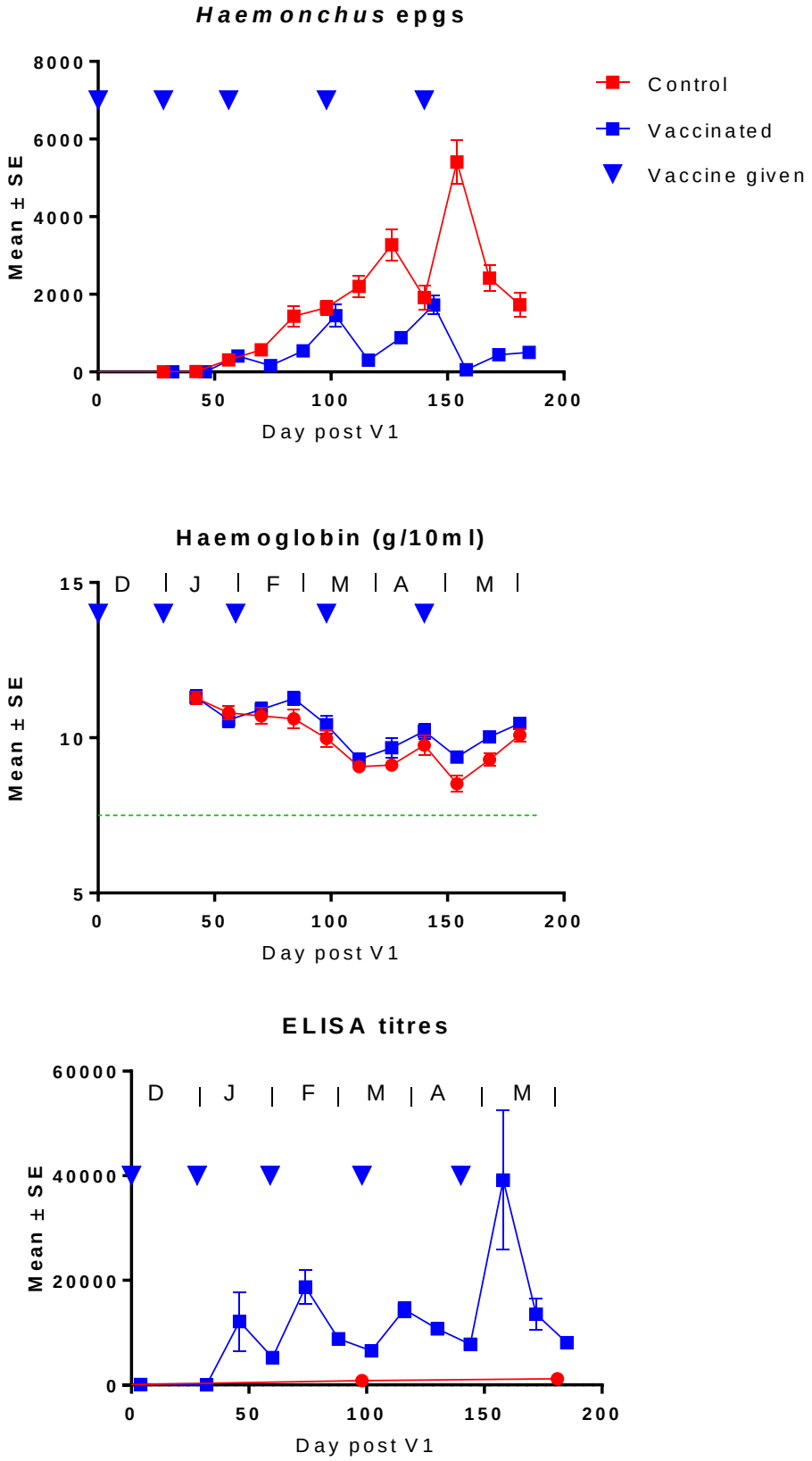

Fig 4. Kinetics of *Haemonchus* egg counts in relation to blood haemoglobin concentrations and anti-vaccine antibody titres.

c. **Blood haemoglobin concentrations:** Means of the two groups of kids are depicted in the central panel of Fig 4. Mean vaccinate concentrations were nearly always slightly lower than control values but not significantly so. Seven control kids required a precautionary drench mostly from Day 140 onwards compared to 4 vaccinate kids which were usually treated earlier in the trial (Table 10, Appendix 3).

d. Antibody concentrations: In the control goats these remained at baseline levels throughout the trial, however those of the vaccinates rose sharply after the second vaccination to a mean of approximately 12,000 (Fig 4 lower panel). Further spikes in titre were observed in the vaccinates following each subsequent boost, but mean titres were always maintained above 5,000.

e. Body Weights: Over the duration of the study the kids gained an average 10.5 kg which indicates that they had maintained a normal growth pattern. Group mean bodyweights are summarized in Table 3. Day -7, Day 70 and Day 181 bodyweights were analyzed for significant differences between groups on each day but no significant differences were detected. Individual animal bodyweights are presented in Table 12, Appendix 3.

Table 4: Summary of Group Mean Bodyweights

Mean Weight (kg) (+/-SD)			
Group	**Day -7**	**Day 70**	**Day 181**
1	11.6[a] (1.7)	19.3[a] (2.8)	22.3[a] (2.9)
2	11.3[a] (1.8)	18.7[a] (2.7)	21.6[a] (2.8)

Entered by T. Dale, Verified by D. Venet

[a] Means in the same row with the same subscript are not significantly different at p≤0.05

36. CONCLUSIONS

SAFETY

It was concluded that Barbervax caused temporary pyrexia a day after it was injected. On average this rise in body temperature was less than one degree centigrade and lasted for only a 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.

The overall conclusion was that the adverse signs associated with administration of Barbervax were mild and commercially acceptable. The data essentially confirmed that previously observed in lambs.

EFFICACY

Overall it was clear that Barbervax provided a significant epidemiological benefit to the kids as their *Haemonchus* egg counts were substantially depressed compared to the controls (Fig 2).

This effect seemed to be mirrored by slightly higher blood haemoglobin concentrations and the need for fewer precautionary drenches although in neither case was the difference

statistically significant. There was no suggestion that Barbervax adversely affected the growth rate of the goats.

Comparison of this data with that from other similar field trials with goat kids and lambs will be made elsewhere.

ABBREVIATIONS

VICH	International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Products
GCP	Good Clinical Practice
APVMA	Australian Pesticides and Veterinary Medicines Authority
MORAG	Veterinary Manual of Requirements and Guidelines
VHR	Veterinary Health Research
SOP	Standard Operating Procedure
WHP	Withholding Period
MSDS	Material Safety Data Sheet
ID	Identification
UNE	University of New England
AEC	Animal Ethics Committee
QA	Quality Assurance
kg	Kilograms
g	Grams
µg	Micrograms
mg	Milligrams
mL	Millilitres
FEC	Faecal Egg Count
SD	Standard Deviation

8.3.3 Near Armidale, NSW

CSIRO Livestock Industries

Field test of Barbervax efficacy for Boer goat kids.

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

Field trial of Barbervax in goat kids.

1. Objective

The trial assesses the efficacy of Barbervax for weaned goat kids. Barbervax is a vaccine for *Haemonchus contortus*, registered for use in Australian lambs.

2. Justification

Haemonchus contortus is the major nematode pathogen of goats in high rainfall areas, more especially as anthelmintic resistant strains are common and widespread. An effective vaccine against this pathogen would be highly desirable for goats and since Barbervax is effective for lambs it seemed reasonable to assume it would likely to be useful for kids as well.

3. Compliance

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

4. Test Site

Animal phase
CSIRO Livestock Industries
Chiswick Field Station
Armidale NSW 2350

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

Antibody analyses
Moredun Research Institute
Pentlands Science Park
Bush Loan
Penicuik
Midlothian
Scotland, UK

5. Study Dates

Start date (Animal Phase):	24 September 2014
Finish date (Animal phase):	11 March 2015
Finish date (laboratory phase):	15 August 2015

6. Study Design

Fifty Boer goat kids (25 castrate male and 25 female) were randomly selected from B. Crouch's herd at "Sentry Box" near Uralla after excessively heavy or light "outliers" had been removed. All trial animals were weighed on Day 0 and ranked from heaviest to lightest within each sex. The 25 animals within each sex were ranked by live weight, sequentially blocked into groups of two (2) animals and randomly allocated, utilizing the "draw from hat" methodology from each of the two blocked groups to form two treatment groups each of 25 animals (Table 1).

One group received the course of Barbervax, the other group was not treated and served as challenge controls.

Kids remained with their mothers until they were weaned and transported to Chiswick on 27 October after which all trial animals were run together as a single mob on a paddock previously grazed by wormy sheep.

Barbervax was given on Weeks 0, 4, 8, 14, 20. Faecal samples for worm egg counts were collected from all kids fortnightly throughout the trial. Blood was collected by jugular venepuncture into 6 mL sodium heparin vacutainers (BD Ltd, Australia) from individual lambs on Weeks 0, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 for haemoglobin concentration estimation (using Haemocue method) and plasma collection.. Laboratory personnel performing FEC and haemoglobin analysis were blinded as to treatment groups.

In addition to the above animals, a third group of 8 age matched kids served as tracers
to determine worm availability on pasture at regular intervals. These animals had faecal samples collected fortnightly for egg counting. On Weeks 6, 12 and 18 they were drenched with Triguard to remove their existing nematode population.

If at any time during the trial, the blood haemoglobin concentration of any animal was <75 g/100mL (equivalent PCV 22%) or its FEC was>10,000, the animal was treated immediately with Hat Trick (or alternative 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 and control products

a. Investigational Veterinary Product (IVP):

Name:	BarberVax	Batch No.:	8
Composition:	*Haemonchus* antigen and saponin adjuvant	Expiry Date:	Oct 2015
Dose Rate:	5µg antigen and 1mg saponin	WHP:	none

b. Source: WormVax 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 was not provided for the IVP.

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

8. Treatment

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

9. Schedule of events

Date	Day	Activity
24 Sept	0	Sentry Box: The owner had had some deaths probably due to *Haemonchus* so only 65 kids enrolled for trial. All drenched with Levamisole / Praziquantel by the owner. All were vaccinated with Orf and Clostridial vaccines and faeces and blood sampled, but Barbervax was given only to the vaccine group. **V1**
23 Oct	28	25 vaccinates, 25 controls and 8 tracers. For Chiswick quarantine reasons, all were given monepantel, a macrocyclic lactone and tricalabendazole. Faeces and blood sampled. **V2**
27 Oct	32	Kids weaned and moved to Chiswick. One vaccinate had a fever, received antibiotics and was removed from the trial.
5 Nov	42	A vaccinate (pneumonia) and a control (accidentally run over by a vehicle) died and.Tracers drenched, all kids faeces and blood sampled.
19 Nov	56	All kids faeces and blood sampled. **V3**
3 Dec	70	All kids faeces and blood sampled
17 Dec	84	Tracers drenched. All kids faeces and blood sampled
5 Jan	103	One vaccinate died - cause unknown. All kids faeces and blood sampled. **V4**
14 Jan	112	All kids faeces and blood sampled
28 Jan	126	Tracers drenched. All kids faeces and blood sampled
11 Feb	140	All kids faeces and blood sampled. **V5**
25 Feb	156	All kids faeces and blood sampled
11 Mar	168	All kids faeces and blood sampled. All drenched with monepantel, rametin, macrocyclic lactone and returned to the owner at Sentry Box.

10. Deaths.

Three goats died during the trial.

11. Animal Management

a. **Animal Welfare:** Study animals were managed with due regard for their welfare. They were observed at least twice weekly for health problems according to AEC requirements. Animals were handled in compliance with CSIRO AEC 11/19 approved 27/10/11, 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 their owner at Sentry Box at the conclusion of the study.

12. 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 dispatch. Replicate 1 plasma samples were dispatched for analysis on ice-bricks

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

13. Assessment of Effects

a. **Kid liveweights**: Animals were weighed at Week 0, 12 and 24 and individual animal liveweights were recorded. Animal weigh scales were checked pre- and post-weighing with calibrated test weights. Liveweights and liveweight change during the study were compared between groups to determine treatment effects, if any, and are detailed in the results section of this report.

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

c. **Faecal worm egg counts and larval differentiations**: Faecal samples were collected at intervals outlined above. Faecal samples were individually labelled with the animal ID. Individual faecal worm egg counts and group bulk larval differentiation were performed. Faecal worm egg counts and larval differentiation were compared to determine treatment effects, if any, and are detailed in the results section of this report.
Note: where an animal received a salvage drench at any point throughout the study, the subsequent FEC sample collected within 14 days of the salvage drench was excluded from group mean FEC calculations.

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

14. Statistical Analyses

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

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

15. Results

a. Deaths

Of the 3 deaths recorded in Sections 9 and 10, one kid was accidentally run over by a farm vehicle, a second died of pneumonia and the cause of death in the third case was not established.

b. Rainfall and larval availability on pasture

October and November were dry months, but more than 50mm rain fell during each of December, January and February. The tracer goat egg counts showed that *Haemonchus* larvae were being picked up from January onwards (Fig. 1).

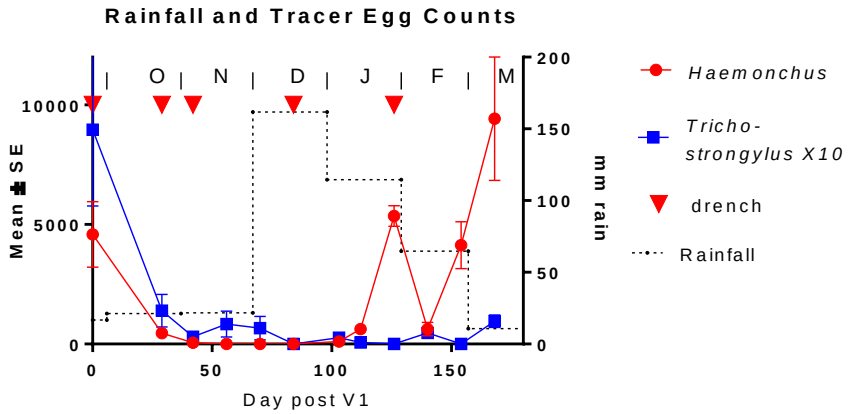

Figure 1. Rainfall and tracer kid egg counts during the trial

Surprisingly high *Haemonchus* and *Trichstrongylus* egg counts were present in some kids at the start of the trial in late September (Table A3 in Appendix 1). These were largely abolished by the drench given at V1 followed by the quarantine anthelmintic treatments given prior to weaning and transport to Chiswick (Fig 3). Counts remained low until late January presumably as a result of the dry spring, but picked up rapidly from early February and remained high for the duration of the trial (Fig 3). *Teladorsagia* were not detected in the coprocultures (Table A5 in Appendix 1), but mean *Trichostrongylus* counts of a few hundred eggs per g were recorded towards the end of the trial (Fig 2).

c. *Haemonchus* Egg Counts

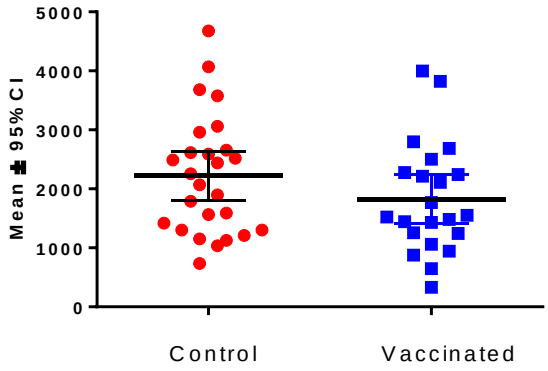

Figure 2. Overall effect of the vaccine on *Haemonchus* egg output from two weeks after V3 (17 Dec) till end of the trial (11 Mar).

There was no significant difference in vaccinate and control egg counts averaged from after the third vaccination to the end of the trial (Fig 2). There appeared to be a partial protective effect from V3, but this disappeared from V5 onwards (Table1, Fig 3).

Table 1. Group mean *Haemonchus* egg counts and vaccination efficacies

Event	Date	Day	Control	Vaccine	%Efficacy
V1	**24/09/2014**	**0**	3870	3575	7.6
V2	**23/10/2014**	**29**	274	285	-4.2
	05/11/2014	**42**	135	76	43.9
V3	**19/11/2014**	**56**	282	142	49.8
	03/12/2014	**70**	216	106	50.9
	17/12/2014	**84**	203	75	63.3
V4	**05/01/2015**	**103**	852	288	66.2
	14/01/2015	**112**	1123	637	43.3
	28/01/2015	**126**	4530	2001	55.8
V5	**11/02/2015**	**140**	4608	4500	2.3
	25/02/2015	**154**	4740	5954	-25.6
	11/03/2015	**168**	4420	3361	24.0

(the earliest the vaccine could have an effect is from day 70)

d. Blood haemoglobin and precautionary drenching

Concentrations below the 75 g/10 ml precautionary threshold were recorded in some 20% (18 out of 58) goats at the first sampling at Sentry box (Table A2 in Appendix 1). Most of these individuals also had high *Haemonchus* egg counts (Table A3 in Appendix 1) implicating the parasite as the likely cause of the anaemia. Thus a total of 20 kids would have required a precautionary drench at the beginning of the trial either because they fell below the pre-set blood haemoglobin threshold, exceeded the egg count limit or both. As it happened all the animals were treated anyway, in accordance with the owner's wishes.

Group mean blood haemoglobins fluctuated around the 100 g/10ml mark for the rest of the trial, no significant differences being recorded between the groups (Fig 4). Several animals in both groups required a precautionary drench in February or March (Table A2 in Appendix 1), coincident with the increase in *Haemonchus* egg counts, but again no differences were apparent between the treatment groups.

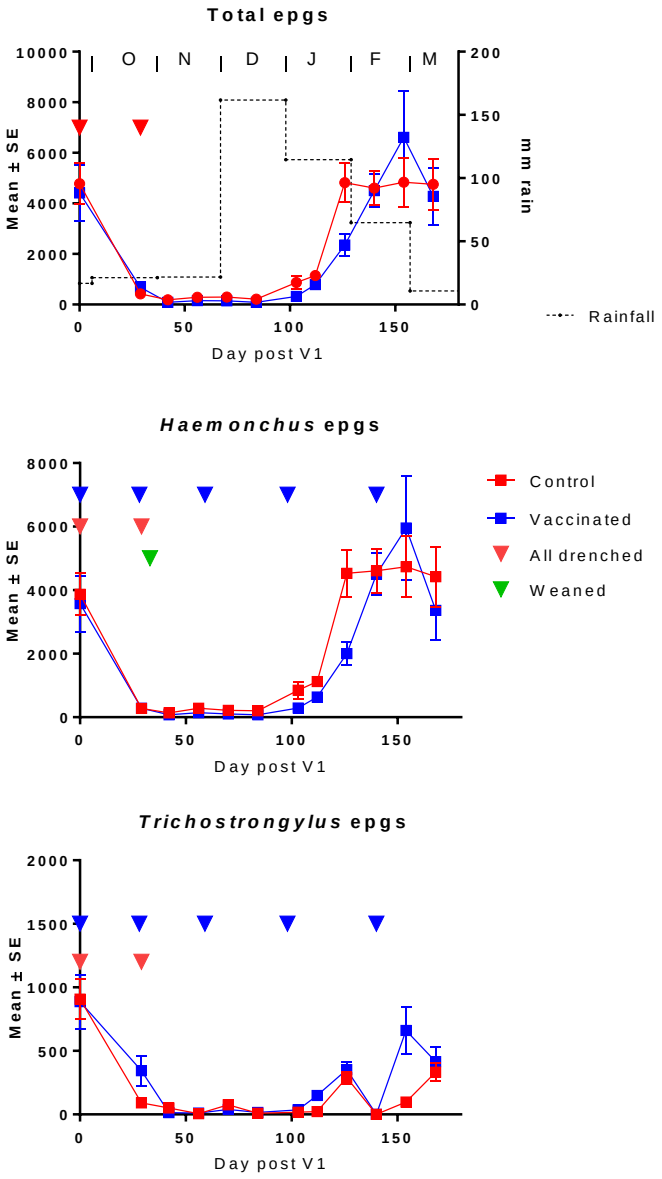

Figure 3. Kinetics of group mean total, *Haemonchus* and *Trichstrongylus* specific egg counts during the trial.

e. Antibody titres

Mean Elisa titres in the controls remained at background levels throughout the trial (Fig 4 lowest panel). In the vaccinated kids, spikes of mean antibody were stimulated after each vaccination and these increased in magnitude after V3 and V4 to reach more than 20,000, before declining to about 15,000 after V5 (Fig 4). Although mean vaccinate titres had declined by 6 weeks after each vaccination from V2, they never fell below 4,900

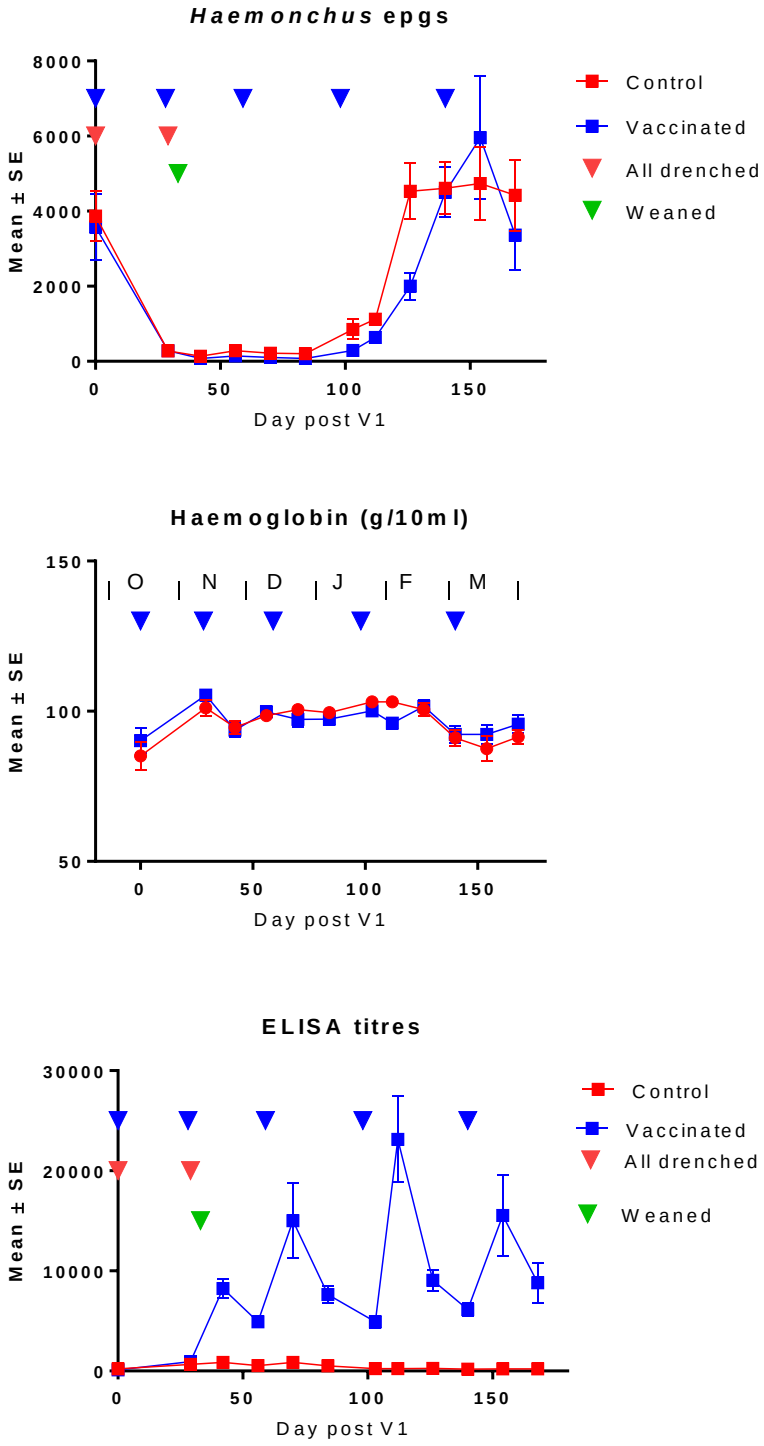

Figure 4. Kinetics of the *Haemonchus* specific egg counts in relation to blood haemoglobin concentrations and antibody titres.

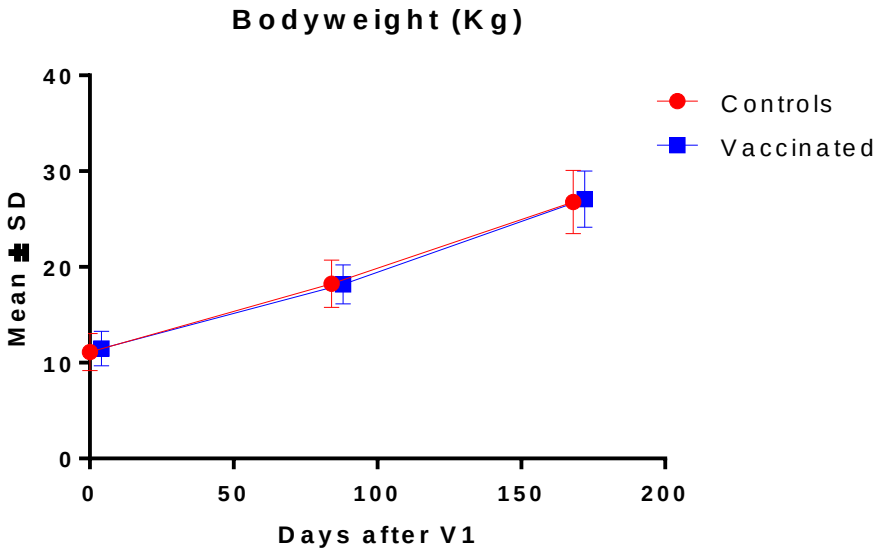

Figure 5. Kid weights during the course of the trial

f. Bodyweights

The kids were weighed at the start, mid way and the end of the trial, growing from a mean of approximately 11 to 27 Kg over the 14 week study period (Table A1 in Appendix 1). Group mean bodyweights are presented in Fig 5 above where it can be seen that there were no significant differences in growth rate.

16. Discussion/conclusions

No significant or useful efficacy in terms of reducing *Haemonchus* faecal egg counts or anaemia was conferred by Barbervax on the kids in the present study. This was despite the vaccine apparently inducing an antibody response of similar magnitude and kinetics to that recorded in previous trials with kids or lambs where useful protection was obtained.

A more detailed analysis will be done to compare the data from the present trial with those from the other two more successful contemporary kid trials and with four previously conducted lamb trials in an attempt to pin point the possible cause of this vaccine failure. That analysis will be described in the final report of the present project.