Duration of protection provided by *Barbervax®*

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Abstract.

An experiment was conducted on a commercial sheep property in the New England region to properly define the duration and level of protection against *H. contortus* in hoggets following a full course of *Barbervax®.* The experiment also aimed to better define the association between antibody levels in response to vaccination and worm egg counts (WEC). A full 2 x 3 factorial design was used with two levels of vaccination and 3 tests of immunity (4, 8 and 12 weeks after final vaccination). 120 Merino ewes approximately 16 months old were used, providing 20 animals per group. Vaccinated animals received four vaccinations 4-5 weeks apart between December 2015 and March 2016, while controls only received the first vaccination. To assess the level of vaccinal immunity animals were artificially challenged with a single dose of *H. contortus* larvae (5,000 L3/ewe) at either 4, 8 or 12 weeks after the final vaccination. Vaccination significantly reduced WECs at 8 weeks after the final vaccination but not at 4 or 12 weeks. Vaccination had no effect on red and white blood cell parameters. Overall antibody levels to the vaccine were higher in vaccinated animals than in controls. In vaccinated animals antibody levels declined over the experiment. No association between antibody titre and WEC was found. The results of the experiment demonstrate *Barbervax®* provided protection at 8 weeks after the final vaccination and that antibody titre is a poor indicator for protection following artificial challenge.

Introduction.

Gastrointestinal nematodes (GIN) cost the Australian sheep industry $436 m per annum, mostly due to production losses (Lane *et al.* 2015). In the summer rainfall areas of Australia, including the New England region, *H. contortus* is the dominant GIN, causing the most damage during late summer and autumn. Adult *H. contortus* consume host blood and

mainly cause harm to the host through blood loss leading to anaemia and loss of protein in the gastrointestinal tract (Parkins and Holmes 1989; Albers *et al.* 1990).

Traditionally control of *H. contortus* has been based on anthelmintics, however widespread anthelmintic resistance is now a major problem. Resistance is considered present when the efficacy of an anthelminthic falls below 95% (Palmer *et al.* 2001). Resistance to all the major classes of anthelmintics has been observed, including monepantel released in Australia in 2010. (Jackson *et al.* 2009; Little *et al.* 2011; Scott *et al.* 2013). Therefore the use of anthelmintics alone is not a sustainable control measure for *H. contortus* and needs to be used in combination with other methods. Integrated parasite management (IMP) combines the use of tactical anthelmintic treatments and non-chemical approaches such as grazing management, genetic selection and vaccination for the control of GIN (Kelly *et al.* 2010).

Vaccination attempts to control the parasite during the host part of the parasite lifecycle. There have been numerous attempts to create a vaccine against *H. contortus* using attenuated larvae and *H. contortus* secretory/excretory proteins (Smith 1999; Bassetto and Amarante 2015). However the hidden antigen approach has been the most successful. This involves using proteins derived from the *H. contortus* intestinal brush border. The sheep's immune system is not naturally exposed to these proteins (Bassetto and Amarante 2015). When the proteins are injected into the sheep, the immune system responds by producing antibodies that circulate in the blood. *H. contortus* ingest the antibodies when they consume the host's blood and these antibodies bind to the proteins in the intestinal brush border and inhibit normal gut function. As a result the parasite starves and has a reduced fecundity (Smith 1999).

Based on this hidden antigen approach, the release of the commercial vaccine *Barbervax®* in 2014, means vaccination against *H. contortus* is now a control option available to sheep producers. In the vaccination schedule previously unvaccinated animals are given three priming vaccinations to develop protective immunity then vaccinations are given at six weekly intervals to maintain protective immunity (Besier *et al.* 2015). The frequency of vaccination is unfavourable for use in a production system but is necessary to

maintain protective immunity. This is because the vaccine is based on a hidden antigen approach and stimulates immunity separate from the immunity developed during natural infection. Therefore exposure to *H. contortus* does not stimulate or boost the immunity provided by the vaccine (Besier *et al.* 2015).

During three registration trials for the use of *Barbervax®* in yearling sheep, worm egg output was reduced by between 64 and 82%, depending on the trial (Smith 2014a). However the trials did not measure the protection provided by the vaccine beyond 2, 5 or 6 weeks after the final vaccination. When WECs were measured at 6 weeks the efficacy of the vaccine was 69.9% (Smith 2014a). This suggests that the vaccine may provide some protection beyond 6 weeks after the final vaccination. These trials did not test for an association between antibody level and WEC.

Properly defining the duration and level of protection against *H. contortus* in hoggets is important for maximising the benefits of vaccination. The following experiment was designed to test two hypotheses. Firstly, that the duration of protection against *H. contortus* provided by a full course of *Barbervax®* is greater than 6 weeks. Secondly, that antibody levels will be significantly correlated with level of protection and thus antibody levels could be used to infer the level of protection.

Materials and Methods.

Experimental design

The experiment utilised a fully randomised complete 2 x 3 factorial design with two levels of vaccination and assessment of immunity by artificial challenge at 3 times after the final vaccination. The experiment was conducted between 4 December 2015 (day -98) and 8 July 2016 (day 119) on a commercial sheep farm, “Congi Station”, Woolbrook, NSW. The two levels of vaccination were: vaccinated, where animals received four doses of *Barbervax®* and control, where animals only received one dose of *Barbervax®* at the beginning of the experiment. Animals were artificially challenged with infective *H. contortus* L3 at either 4, 8 or 12 weeks after the finial vaccination. The day of the 4th and final vaccination was considered as day 0 of the experiment. There were 6 groups in total (Table

1), with 20 animals in each group, 120 animals in the experiment. Animals were randomly assigned to groups as they came through the race. Individual animal was the experimental unit, meaning each treatment combination had 20 replicates.

Table 1: Experimental groups, numbers and treatments

Group	Number of Animals	Treatment	Challenge week post completion of vaccination course
1	20	Vaccinated	4
2	20	Control	4
3	20	Vaccinated	8
4	20	Control	8
5	20	Vaccinated	12
6	20	Control	12

Animal management

The experiment was approved by the University of New England Animal Ethics Committee (AEC15-131). Animals used were fine wool Merino hoggets approximately 16 months old that the start of the experiment. Animals were managed as a single mob on "Congi Station", Woolbrook, NSW by the station manager. Woolbrook has summer dominant rainfall with an annual average rainfall of 780 mm. Average temperature range in summer is between 12°C and 26°C and in winter between -1° to 13°C. Animals were individually identified with visual and electronic ear tags. All of the animals received a full course of Barbervax® as lambs in the previous year. On 9 February 2016 the average ewe weight was 44.0 kg (range 33.0 – 55.5 kg). Animals were grazed on native pastures, however due to a dry autumn the manager began supplementary feeding of whole barley at the start of May. The introductory ration was 100g barley/ewe three times per week which was slowly increased to 1kg barley/ewe three times per week. Feeding continued for the remainder of the experiment. Ewes were joined between 14 April 2016 and 25 May 2016. No animals died during the experiment.

Application of treatments

Animals were drenched 7 days before each artificial challenge and again 35 days after challenge to remove infection. Drenching involved the oral administration of 12mL of *Startect®* (active ingredients: 10g/L derquantel and 1 g/L abamectin) per animal. Precautionary anthelmintic treatments in response to rising WECs were given to all animals at the 3rd vaccination (day -35) and at the week 4 challenge (day 56) to groups 3, 4, 5 and 6 (Fig. 1). The vaccine used was *Barbervax®* (batch 11/1; expiry date February 2017; Wormvax Australia Pty Ltd, Western Australia) which contains purified *H. contortus* antigen (5µg/mL). Vaccinations were given at 4-5 weekly intervals with 4 vaccinations in total and the vaccine was kindly provided by Dr David Smith (Moredun Research Institute, Scotland). *Barbervax®* was administered subcutaneously at a dose rate of 1mL per animal. Artificial infection involved orally administering fully drench susceptible L3 of *H. contortus* (Kirby strain) supplied by Veterinary Heath Research (Armidale, NSW). The dose rate was 5000 L3 per animal in 4mL of tap water. Animals were challenged at either 4, 8 or 12 weeks post final vaccination (Fig.2).

Fig. 1: Application of treatments and timeline of the experiment

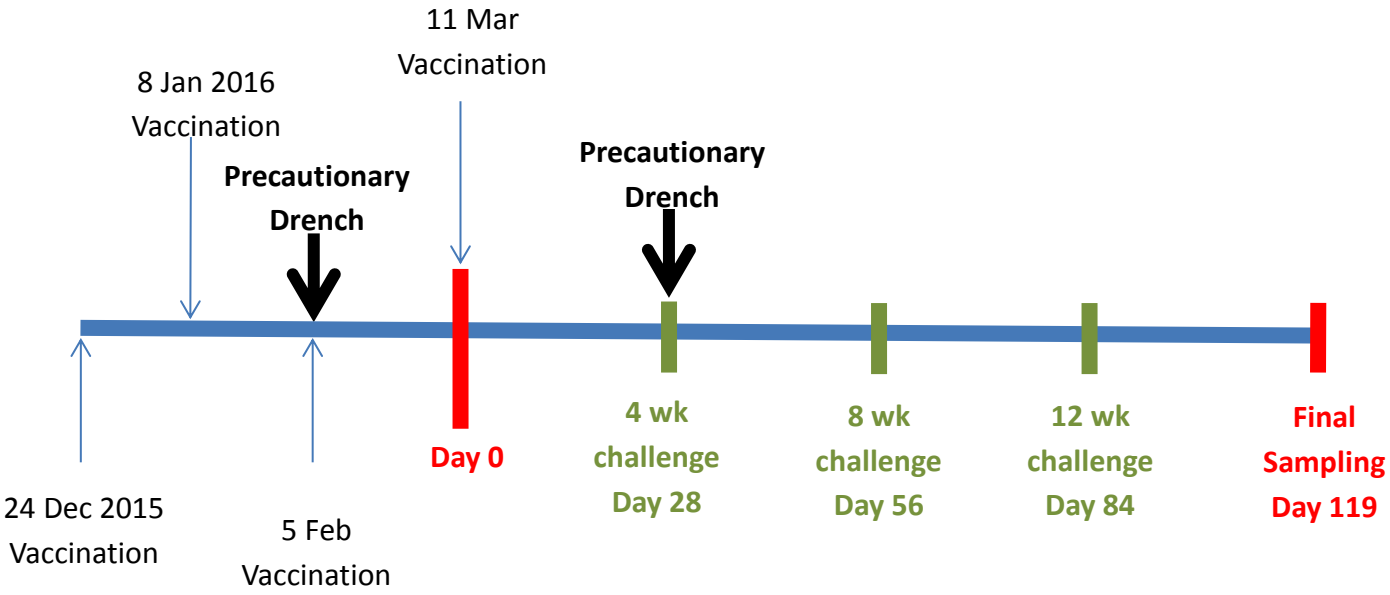
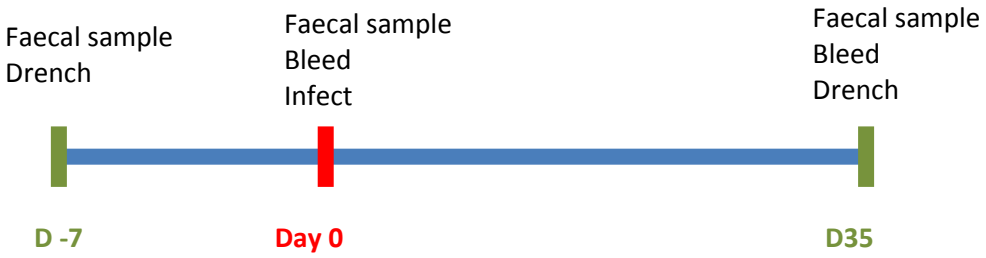


Fig. 2: Timing of sample collection and treatments during the 4, 8 and 12-week artificial challenges.



Measurements schedule and collection of samples

Rectal faecal samples were collected on day -7, 0 and 35 relative to challenge for WECs and larval differentiations. The day -7 samples were to give an indication of natural challenge, day 0 was to confirm the pre-artificial challenge was effective and the day 35 as a measure of immunity in response to challenge. A separate glove was used for each individual animal and faecal samples placed separate containers. Background WECs were taken at the time of the 3rd and 4th vaccination, with 15 samples taken at random from both the vaccinated and unvaccinated groups. Blood samples were taken from the jugular vein using an 18 gauge needle and holder in a 6mL K2EDTA tubes and immediately placed on ice. Samples were collected from each animal on days 0 and 35, relative to the challenge date.

Laboratory methods

Worm Egg Counts (WECs).

Approximately 2 grams of the faecal sample was diluted with distilled water 1:5 weight to weight with an automatic diluter made by the Science and Engineering Faculty at UNE. The samples stored 4°C for no more than 3 days. Then the samples were grounded to form a homogenous liquid and sieved. 600µL of saturated salt solution and 150µL of faecal solution was placed in the well of a 0.5mL Whitlock chamber slide. Eggs were counted under the microscope at 40x magnification with one egg corresponding to 60 eggs per gram of faeces.

Larval differentiation.

The remaining faecal sample was pooled within each group and combined to form a thick paste using tap water and vermiculite in a glass jar. The samples were incubated at

approximately 24°C for 7 days at 100% humidity. After 7 days the jar was filled with tap water until the meniscus could be seen. The jar was inverted onto a glass petri dish and the dish filled with tap water. After at least one hour the larval were drawn off the edge of the petri dish. One drop of larval solution and one drop of iodine solution were put on a slide. 100 larval were counted and identified as either *H. contortus* or other, based on tail length and head shape, under a light microscope.

Haematology.

Blood samples were analysed using an automated haematology analyser (CellDyn 3700; Abbott Diagnostics) calibrated for sheep blood on the day of collection at CSIRO Chiswick laboratory (Armidale, NSW). White blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin concentration and platelets were measured.

Antibody titres.

After haematology analysis samples were then spun in a centrifuge (Beckman Allegra X-15R) at 2 500 RPM at 4°C for 15 minutes and the serum was drawn off and frozen until analysed for antibody levels. Serum antibodies produced in response to vaccination with *Barbervax®* were measured using enzyme linked immunosorbent assay (ELISA). Antigen used to manufacture of *Barbervax®* was kindly provided by Dr David Smith (Moredun Institute) and diluted in sodium carbonate coating buffer (pH 9.6) to a concentration of 1µg antigen/mL of buffer. 50µL of solution was added to each well on 96 well high binding plates and incubated overnight at 4°C. Plates were blocked and incubated over night at 4°C with a solution containing 10% (w/v) infant soy milk powder (Karicare+®) dissolved in Tris/HCl, NaCl, Tween 20 and thimerosal (TNTT). Serial doubling dilutions of test serum were prepared in a low binding ELISA plate from 1:200 to 1:204,800. All dilutions were in TNTT. 50µL of each diluted sample was transferred to the high binding plate and incubated at room temperature for 1 hour. Secondary antibody, mouse monoclonal anti-goat/sheep IgG-HRPO conjugate (Sigma) diluted to 1/10,000 in TNTT was added to each well and incubated for 1 hour at room temperature. 50µL o-phenylenediamine dihydrochlorie (OPD) dissolved in distilled water was added to each well. Between each step plates were washed with

phosphate buffered saline contain 0.5% v/v Tween 20 (PBST). The reaction was stopped after 20 with the addition of 20µL 2.5M sulphuric acid. Plates were read at 490 nm using an ELISA plate reader (Benchmark). Titres were calculated using the method developed by Moredun Institute. The curvilinear relationship of natural log dilution and absorbance was plotted. The titre was considered where the curve intersected the negative control. Dilations between 1:200 and 1: 12800 were used in the calculations.

Non-responders to the vaccine

Non-responders to the vaccine were considered as either falling within control upper 95% confidence interval for log10 antibody titre or within the lower 95% confidence interval for cube root WEC. Analysis was done within time period.

Statistical analysis

Data were analysed using JMP® 12.1.0 (2015, SAS Institute Inc.). WEC data were cube root transformed and antibody titres were log 10 transformed to meet the assumptions of the analysis. One-way analysis of variance was used to test the effect of vaccination treatment within time period for WEC data. For analysis of antibody titre the effects of vaccination treatment, time of challenge and their interaction were tested using analysis of variance. To test the association between WEC and antibody titre a spline-smoothed curve was fitted to determine the underlying shape of association within each time period and overall. As the underlying shape was not curvilinear, a linear regression was then fitted to test the strength of the association. Least squares means and standard errors are reported.

Results

Worm egg counts

Day -7 pre-challenge WECs resulting from natural exposure to larvae from the pasture were numerically lower in the vaccinated animals compared to the controls throughout the experiment (Fig. 3). However this difference was only significant in the cube root transformed WECs at days 0 (P=0.0272) and 21 (P=0.0007). The raw WEC data from

natural challenge shows a large difference (>1400 epg) between treatments at week -35, although this was not significant (Fig. 4).

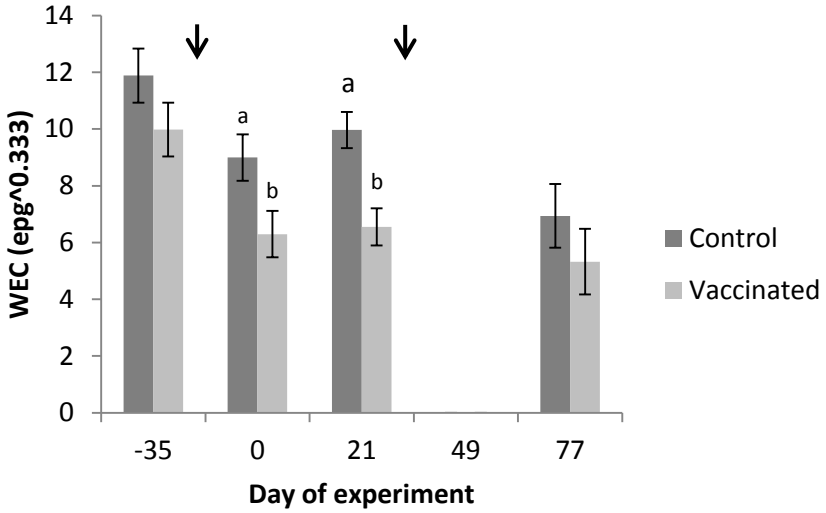


Fig. 3. Cube root transformed worm egg counts (eggs per gram) throughout the experiment in response to natural infection from the pasture in vaccinated and control animals. ↓represents precautionary drenches. Column with different letters within week differ significantly (P≤0.05).

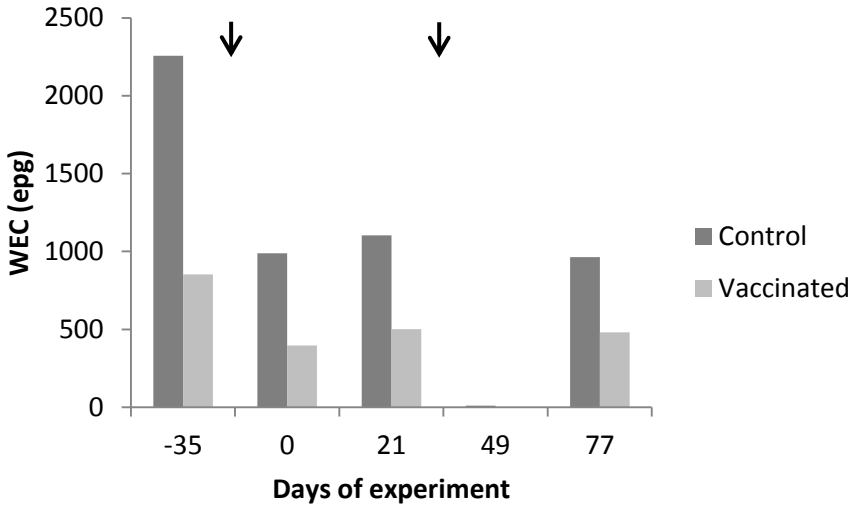


Fig. 4. Raw untransformed worm egg counts (eggs per gram) throughout the experiment in response to natural infection from the pasture in vaccinated and control animals. ↓represents precautionary drenches.

Cube root transformed WECs were numerically lower in the vaccinated animals at both weeks 4 and 8 after artificial challenge (Fig. 5) but only at week 8 was the difference significant (P=0.05). The raw data shows a large difference between control and vaccinated WECs at week 4 (Fig. 6) despite the lack of statistical significance.

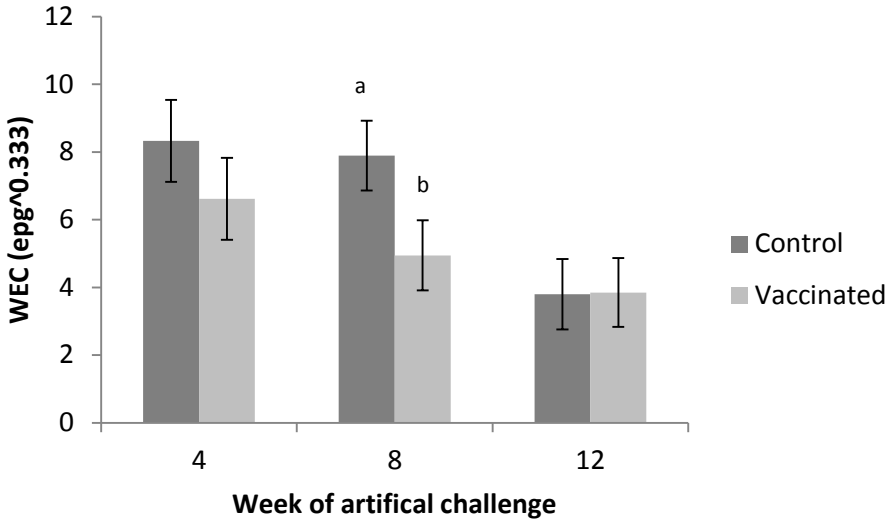


Fig. 5. Cube root transformed worm egg counts (eggs per gram) throughout the experiment in response to artificial infection in vaccinated and control animals. Column with different letters within week differ significantly (P≤0.05).

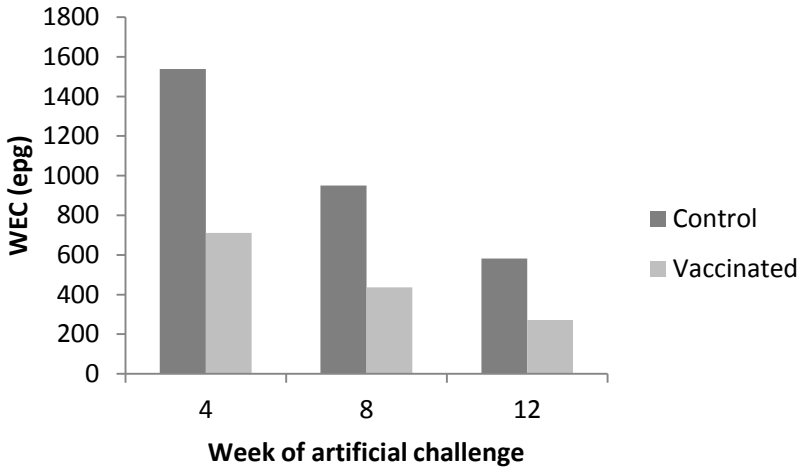


Fig. 6. Raw worm egg counts (eggs per gram) throughout the experiment in response to artificial infection in vaccinated and control animals.

Larval differentiation

There was no significant difference in the percentage of *H. contortus* larvae between vaccinated and control animals during the experiment. The percentage of *H. contortus* in vaccinated animals ranged between 93 – 100%, in the controls it ranged between 91.5 – 100% (Table 2).

Table 2: Percentage of *H. contortus* L3 in larval differentiation through the experiment.

Day of experiment	Group	Treatment	% *H. contortus*	% Other	Notes
-35	-	vaccinated	96.5	3.5	3rd vaccination
	-	control	94	6	
0	-	vaccinated	100	0	4th vaccination
	-	control	100	0	
21	1	vaccinated	100	0	Pre-challenge
	2	control	99.5	0.5	drench
49	3	vaccinated	100	0	Pre-challenge
	4	control	99	1	drench
63	1	vaccinated	98.5	1.5	Day 35 after
	2	control	99	1	challenge
77	5	vaccinated	97	3	Pre-challenge
	6	control	97	3	drench
91	3	vaccinated	93	7	Day 35 after
	4	control	91.5	8.5	challenge
119	5	vaccinated	98.5	1.5	Day 35 after
	6	control	97	3	challenge

Haematology

Vaccination had no effect on the following red blood cell parameters; red blood cell count, haemoglobin and packed cell volume before or after artificial challenge at 4, 8 or 12 weeks. There was also no effect of vaccination on the white blood cell parameters; white blood cell count, neutrophils, lymphocytes, monocytes, eosinophils, basophils or palettes before or after artificial challenge at 4, 8 or 12 weeks (Table 3).

Table 3: Haematology before (D0) and after (D35) artificial challenge at 4, 8, or 12 weeks after the final vaccination.

		Week 4 Challenge		Week 8 Challenge		Week 12 Challenge		Normal range for sheep *
		Control	Vaccinate	Control	Vaccinated	Control	Vaccinated	
D0 white blood cells (10*6/L)	Mean	5.55	5.88	6.58	6.26	5.82	6.46	4.0-12.0
	s.e	0.21	0.31	0.27	0.37	0.29	0.37	
D0 neutrophils (10*6/mL)	Mean	2.28	2.33	2.96	2.61	2.49	2.97	0.7-6.0
	s.e	0.14	0.22	0.21	0.18	0.18	0.26	
D0 lymphocytes (10*6/mL)	Mean	2.55	2.81	2.71	2.76	2.46	2.60	2.0-9.0
	s.e	0.14	0.20	0.17	0.23	0.17	0.21	
D0 monocytes (10*6/mL)	Mean	0.42	0.45	0.54	0.59	0.57	0.58	0.0-0.8
	s.e	0.04	0.07	0.09	0.10	0.07	0.07	
D0 eosinophils (10*6/mL)	Mean	0.21	0.20	0.25	0.20	0.20	0.21	0.0-1.0
	s.e	0.04	0.02	0.04	0.02	0.03	0.03	
D0 red blood cells (10*6/mL)	Mean	9.99	9.29	10.30	9.94	9.27	9.36	9.0-15.0
	s.e	0.22	0.19	0.17	0.22	0.25	0.17	
D0 haemoglobin (g/dL)	Mean	10.86	10.59	11.05	10.94	10.79	10.79	9.0-15.0
	s.e	0.22	0.21	0.18	0.22	0.22	0.18	
D0 packed cell volume (%)	Mean	28.77	28.67	32.53	32.61	28.74	28.99	27-45
	s.e	0.54	0.51	0.49	0.51	0.50	0.47	
D0 mean corpuscular volume	Mean	28.88	31.00	31.69	32.98	31.27	31.05	28-40
	s.e	0.38	0.64	0.58	0.67	0.57	0.51	
D0 mean corpuscular	Mean	10.89	11.44	10.75	11.04	11.71	11.54	8.0-12.0
	s.e	0.11	0.20	0.17	0.20	0.16	0.15	
D35 white blood cells (10*6/L)	Mean	6.14	6.01	6.03	5.78	6.10	6.44	4.0-12.0
	s.e	0.29	0.28	0.36	0.31	0.32	0.42	
D35 neutrophils (10*6/mL)	Mean	2.63	2.60	2.70	2.28	2.19	2.52	0.7-6.0
	s.e	0.13	0.20	0.30	0.25	0.17	0.36	
D35 lymphocytes (10*6/mL)	Mean	2.34	2.16	2.25	2.48	2.74	2.88	2.0-9.0
	s.e	0.14	0.14	0.17	0.21	0.22	0.19	
D35 monocytes (10*6/mL)	Mean	0.71	0.78	0.62	0.53	0.63	0.63	0.0-0.8
	s.e	0.10	0.09	0.08	0.09	0.07	0.08	
D35 eosinophils (10*6/mL)	Mean	0.36	0.37	0.33	0.37	0.28	0.29	0.0-1.0
	s.e	0.04	0.06	0.05	0.06	0.04	0.04	
D35 red blood cells (10*6/mL)	Mean	9.96	9.63	9.26	9.35	9.53	9.43	9.0-15.0
	s.e	0.18	0.21	0.17	0.22	0.17	0.17	
D35 haemoglobin (g/dL)	Mean	10.90	10.50	10.52	10.70	11.13	10.91	9.0-15.0
	s.e	0.17	0.21	0.14	0.23	0.17	0.20	
D35 packed cell volume (%)	Mean	31.31	30.91	28.61	28.96	29.57	29.29	27-45
	s.e	0.58	0.62	0.33	0.54	0.42	0.51	
D35 mean corpuscular volume	Mean	31.51	32.16	31.03	31.05	31.13	31.10	28-40
	s.e	0.60	0.41	0.49	0.36	0.47	0.41	
D35 mean corpuscular	Mean	10.98	10.92	11.40	11.45	11.70	11.58	8.0-12.0
	s.e	0.20	0.14	0.15	0.08	0.13	0.13	

*Normal range from Radostits *et al.* (2007).

ELISAs

Analysis of antibody titres showed both the main effects of treatment and time were significant, P values were much less than 0.05. The effect of vaccination was significantly influence by week after the final vaccination, the interaction was significant (P=0.05). Antibody titres in the vaccinated animals decreased over time with the greatest decrease between weeks 4 and 8 (Fig. 7). There was a decrease in antibody level between week 8 and 12 in the controls.

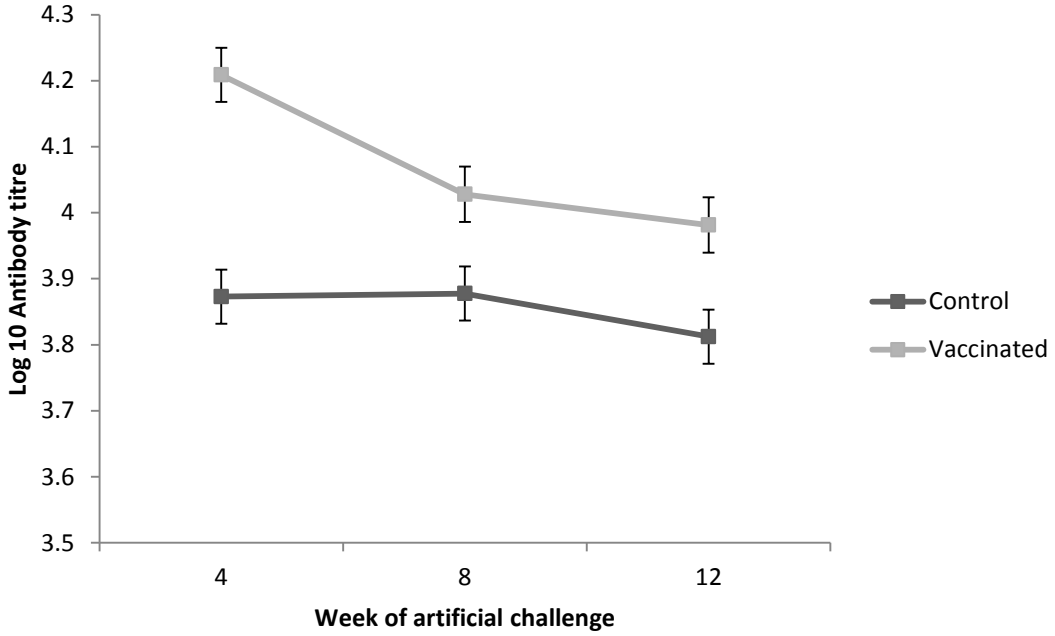


Fig. 7. Log 10 antibody titres (l.s.m ± s.e) showing interaction between the effects of vaccination treatment and challenge time.

Non-responders to the vaccine

The percentage of non-responders based on antibody titre was 10% four weeks after the final vaccination (Table 4). That the same time the percentage non-responders base on WEC was much higher (50%). At 8 and 12 weeks after the final vaccination the percentage of non-responders was higher than at 4 weeks for both antibody titre and WEC.

Table 4. Number and percentage of non-responders to the vaccine based on antibody titre and WEC 4, 8 and 12 weeks after the final vaccination.

	Antibody titre	WEC
week 4	2 (10%)	10 (50%)
week 8	5 (26%)	8 (42%)
week 12	3 (16%)	12 (63%)

Relationship between antibody titre and WEC

The variation in WEC was not explained by antibody titre. When cube root WEC was fitted as a response to log 10 antibody titre there was no significant linear relationship (P=0.35, $R^2 < 0.01$) although, the spline (λ=100) fit indicated a linear rather than a curvilinear association. Analysing within time period or with treatment did not improve the linear R-squared value (Fig. 8).

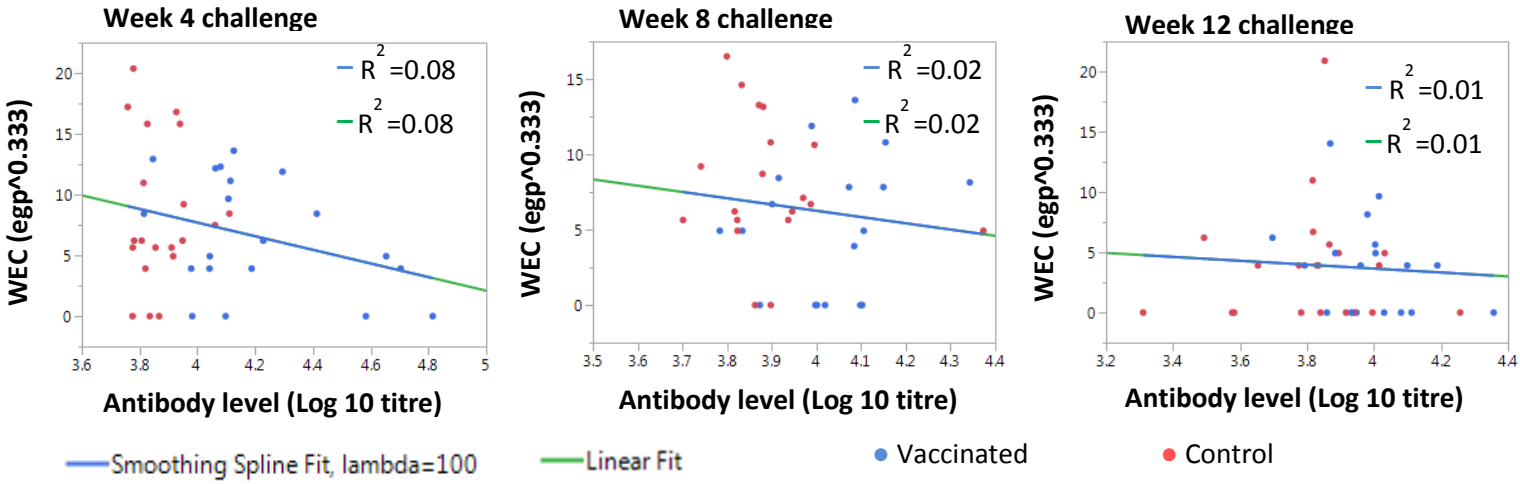


Fig. 8. Relationship between antibody level and WEC with in challenge time.

Discussion

The results of this experiment support the hypothesis that the duration of protection provided by a full course *Barbervax*® vaccinations in year 2 can be greater than 6 weeks. However the results did not support the hypothesis that antibody titres would be significantly correlated with the level of protection.

Vaccination reduced WEC 8 weeks after the final vaccination when animals were artificially challenged. Indicating that vaccination assisted animals in reducing the

establishment of *H. contortus* L3, reducing the fecundity of female worms and/or, causing the death of worms. LeJambre *et al.* (2008) estimated the protection provided by booster vaccinations to be around 7 weeks. However the vaccination contained 100µg each of H11 and H-gal-GP antigen, whereas *Barbervax®* only has 5µg of a mixture of gut proteins. During natural infection the effect of vaccination was significant 3 weeks after the final vaccination. This was in agreement with the two trials undertaken during the registration process where protection was measured at 5 and 6 weeks after the final vaccination and animals were exposed to natural challenge (Smith 2014a). However the effect of vaccination at the 4-week challenge was not significant in the current experiment. This was unexpected given an effect was observed 8 weeks after the final vaccination. However the raw data shows a large difference of over 1400 epg between the treatments. In a farming situation this difference would have a large impact on pasture contamination with *H. contortus* larvae. Preparing clean paddocks for weaners and peri-parturient ewes is an important management strategy for GIN control in susceptible classes of livestock (Bailey *et al.* 2009).

Given the large amount of variation in the WECs measured in this experiment, potentially the power to detect true differences between treatments was insufficient. Each treatment had 20 replicates however in the vaccination registration trials the treatment groups ranged from 30 to 40. Our experiment was conducted on a commercial property with animals that were part of a commercial flock and resources were not unlimited. In previous experiments major effects on WEC in sheep are typically observed when n=20 or less (Datta *et al.* 1998; Datta *et al.* 1999) therefore, using n=20 in our experiment was not unreasonable.

During the experiment vaccinated and control animals were grazed together. However the manufacturer of *Barbervax®* recommends that vaccinated and unvaccinated animals are grazed separately because of the epidemiological consequences of vaccination which lead to reduce pasture contamination by vaccinated animals compared to unvaccinated animals. However during the registration trials with yearlings unvaccinated and unvaccinated animals were grazed together and exposed to L3 on the pasture (Smith 2014a). In this experiment artificial challenge was used to test the level of immunity and WEC was measured at 35 days. Given that it takes 18 to 21 days for L3 ingested from

pasture to mature to egg laying females, there would have only been a 14-17 days of exposure to natural infection during the artificial challenge. It is unlikely that the amount L3 ingested during this time would significantly influence WECs measured at day 35 after artificial challenge. Therefore grazing animals together should not have had a significant impact on the results of artificial challenge in this experiment. However if the two groups were grazed separately and infection rates were modelled based on faecal contamination of pasture greater differences in WECs may be found.

There was no effect of vaccination on blood parameters before or after challenge at any of the challenge times. A possible reason for this is in our experiment the dose of L3 was low (5000 L3/ewe). Le Jambre (1995) modelled the association between WEC and blood loss in sheep infected with *H. contortus* and our results wherein in agreement with this study. Working on a commercial farm we were unable to monitor sheep health daily and wanted to be conservative with dose of L3. The manager may have terminated the experiment if sheep started dying from artificial infection of *H. contortus.* The effect of vaccination on blood parameters may have been greater if the artificial challenge dose was higher given that high WECs are associated with blood loss (Le Jambre 1995).

The larval differentiation showed that throughout the experiment animals were predominantly exposed to *H. contortus* and vaccination had no effect on the percentage of worm species. This was unexpected because if one species of worm was suppressed it would be likely that the proportion of other species would increase. However during our experiment broad spectrum anthelmintics were used, which would have reduce the population of all GIN species. This is a possible reason why the percentage of *H. contortus* remained similar in both treatment groups.

Vaccination significantly increased antibody titres during the experiment. Between challenge times titres in the controls were not significantly different. However with the vaccinated animals a significant declining trend was observed. This confirms the work done in the vaccination registration trials where titres decreased after each vaccination and only increased when booster vaccinations were given (Smith 2014a). This is to be expected because the immunity provided by the vaccine is different to the immunity developed from

natural exposure to *H. contortus.* As a result immunity provided by the vaccine is not stimulated or booster by exposure to *H. contortus.*

Antibody levels in response to vaccination were not correlated to WEC and therefore cannot be used to infer level of protection in individual animals based on WECs. This means that in this experiment antibody titre did not explain the variation in WEC. In contrast, LeJambre *et al.* (2008) found WECs were inversely correlated with antibody titres, however titres were quite variable between animals. The experiment used natural challenge and the vaccination contained 100µg each of H11 and H-gal-GP. During the registration trials in lambs the percentage of non-responders was estimated to be 3% based on WEC values (Smith 2014b). This is much lower than the non-responder percentage based on WEC calculated in our experiment. Possibly high percentage of non-responders may explain the lack of association between antibody level and titre.

Conclusion

In conclusion, *Barbervax®* provided protective immunity for up to 8 weeks and antibody levels were a poor indicator of WECs in this experiment. Further research is required to confirm the duration of protection with more replicates in each treatment group. Potentially this could lead to changes in the use of *Barbervax®* and even label changes. If the number of vaccinations can be reduced or the length between vaccinations increased, this would be of benefit to producers.

Acknowledgments

We thank Madeleine Broomfield for technical support in the field and David Paull at CSIRO for assisting with the Cell-Dyn. We also thank Anthony Uren for allowing us to conduct the experiment at “Congi Station” and managing the animals. The Australian Wool Education Trust provided the funding for this project.

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