Efficacy evaluation of a *Haemonchus contortus* antigen-containing vaccine (Wirevax®) in sheep in South Africa

By

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SUMMARY

Efficacy evaluation of a *Haemonchus contortus* antigen-containing vaccine (Wirevax®) in sheep in South Africa

By

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This study was conducted to confirm the protective properties of the Moredun *H. contortus* antigen-containing vaccine Wirevax® administered to protect sheep against *H. contortus* infection by demonstrating that faecal egg counts and worm burdens are significantly lower in vaccinated animals compared to non-vaccinated animals. Serum samples were collected and antibody titres were measured. It was expected that the protective effect of the vaccine would last at least four weeks after the last dose of vaccine had been administered.

After three vaccinations, given three weeks apart, the experimental vaccine Wirevax® stimulated a strong circulating antibody response. Statistically the mean antibody titres of the vaccinated sheep were significantly higher than those of the control sheep. The immune response was associated with reductions in faecal egg counts and worm burdens in vaccinated sheep when compared to unvaccinated control sheep. After the third vaccination, the efficacy of the vaccine was 86.93% and faecal egg counts were reduced by 95.59%. Thirty days after the last vaccination, the efficacy of the vaccine was 80.66% and faecal egg counts were reduced by 80.6%.

It can be concluded that three vaccinations given at three-week intervals will aid in the control of *H.contortus* infections in sheep and will prevent new infections for a period of 30 days. Statistically, body weight did not correlate significantly with vaccination.

Chapter 1
INTRODUCTION

The intensification of livestock production is an important reason for the increase in morbidity and mortality caused by gastrointestinal parasites (Emery & Wagland 1991). The parasite with the most severe impact on small stock farming worldwide, and also in South Africa, is the haematophagous nematode *Haemonchus contortus* (Miller & Horohov 2006). Depending on the size of the worm burden, infection with this helminth can clinically manifest as a rapidly developing anaemia with oedema, inappetence, loss of condition and death. Unfortunately control still relies to a large extent on the use of anthelmintics. However, since widespread anthelmintic resistance affects almost the entire spectrum of anthelmintic groups, there is an urgent need to develop effective and practical alternative control approaches (Van Wyk, Malan & Randles 1997). Vaccination is one option that, unlike other alternative approaches, offers the advantage that it will stimulate or boost the immune system, resulting in the induction of protective immunity (Knox & Smith 2001). An effective vaccine to control *H. contortus* would benefit the small-stock industry in South Africa and worldwide as it could minimise reliance on chemical drugs. The protective antigens *Haemonchus* galactose-containing glycoprotein complex (H-gal-GP) and H11 have been isolated from *H. contortus* and are the active ingredients in a vaccine that has been commercialised as Wirewax® (Knox & Smith 2001; Smith, Van Wyk & Van Strijp 2001). This efficacy trial conducted in South Africa was undertaken for registration purposes.

Chapter 2
LITERATURE REVIEW

Vaccination against helminths is a control concept that is currently being successfully applied in the control of *Dictyocaulus viviparus* (lungworm) infection in cattle in some parts of the world. Similar attempts have been made to develop vaccines against several economically important gastrointestinal nematodes in small stock and cattle (Ekoja & Smith 2010; Emery & Wagland 1991; Halliday & Smith 2011; Miller & Horohov 2006).

The vaccine strategy that currently promises the most success largely ignores the mechanisms of natural immunity and attempts to direct responses towards external antigens (somatic antigens) on the outer surface (surface antigens) and in excretions and secretions (excretory-secretory antigens, ES antigens) of helminths. Candidate-protective antigens have so far been identified on the surface of oncospheres from *Taenia* and *Echinococcus* sp, and excretions and secretions of *Fasciola hepatica, H. contortus* and *Ostertagia ostertagi* (Smith & Zarlenga 2006).

In the case of blood-feeding *Haemonchus* sp, the luminal surface of the intestine has been a particularly rich source of suitable target molecules (Smith & Zarlenga 2006).

In the most promising attempt to develop a vaccine against *H. contortus* in small stock, highly protective intestinal cell-membrane antigens, namely H-gal-GP and H11, have been identified and isolated at the Moredun Research Institute (Emery & Wagland 1991; Knox & Smith 2001; Smith 2008).

2.1 Preliminary experiments with native antigen

Numerous trials conducted over the past 20 years have demonstrated that the native proteins derived from intestinal cells of adult *H. contortus* can be used to successfully vaccinate sheep against *H. contortus* (Knox & Smith 2001; Knox *et al.* 2003; Smit & Zarlenga 2006). The antigen preparations tested in trials included crude homogenates or detergent extracts of nematode intestines, their membranes, and proteins purified by chromatography (Smith 1993; Jasmer *et al.* 1993; Smith *et al.* 1993). The two protective antigens that have been best characterised and are the most effective are known as H11, which consists of several microsomal aminopeptidases, and H-gal-GP, which is a complex

containing mainly protective aspartyl and metallo proteases (Smith *et al.* 1997; Smith *et al.* 1999; Smith *et al.* 2003a and 2003b).

These two antigens can be prepared simply and cost-effectively by lectin chromatography from a detergent extract of *H. contortus* membranes (Smith *et al.* 1993). Although H11 and H-gal-GP account for 80-90% of the detergent extract, other protective proteins are also present (Smith & Smith 1993; Smith *et al.* 2000; Personal communication with WD Smith 2014).

The other less abundant and characterised protective membrane-bound antigens are P1 or H45, thiol-binding proteins, GA1, P46, P52 and H-sialgal-GP (Smith *et al.* 1993; Knox *et al.* 1999; Jasmer *et al.* 1996; Smith *et al.* 2000).

2.2 H-gal-GP and H11 in perspective

Haemonchus galactose-containing glycoprotein complex (H-gal-GP) binds selectively to lectins and has a specificity for N- acetylgalactosamine. In each experiment, immunisation with H-gal-GP significantly reduced faecal egg counts (FECs) and wireworm burdens in vaccinated sheep compared to the unvaccinated controls. Immunisation was also significantly more effective against female than against male worms, as shown by the abnormal sex ratio of the populations recovered from each vaccinated group (Smith *et al*. 1999).

H-gal-GP is an integral membrane complex of proteases derived from the microvilli of *H. contortus* intestinal cells. It is hypothesised that in vaccinated sheep, H-gal-GP antibodies ingested with the blood meal interfere with the worms' digestion and leads to starvation of the parasites (Smith 2007).

H11 is an integral membrane glycoprotein complex expressed exclusively in the intestinal microvilli of the parasitic stages. It is a highly effective immunogen against *H. contortus* challenge and has resulted in a 90% reduction of faecal egg counts and 75% reduction of worm burdens in vaccination trials (Newton & Munn 1999). The protection is closely correlated with systemic IgG titres to H11 (Munn *et al*. 1997). H11 has several desirable properties. It is an effective immunogen in young lambs (Tavernor *et al*. 1992) and is effective in a range of sheep breeds and against anthelmintic-resistant *H. contortus* strains (Newton & Munn 1999).

The vaccine antigens (H11 and H-gal-GP) are 'hidden antigens', since the immune systems of small stock is not normally exposed to antigens of the luminal surface of *H. contortus* intestinal cells (Smith 1993). The immunity conferred by the vaccine does not interfere with the development of natural immunity (Smith & Smith 1993), but rather provides protection when the latter is acquired (Le Jambre *et al.* 2008).

2.3 Characteristics of the protective immunity conferred by vaccination with *H. contortus* gut membrane proteins

Strong evidence has been found that the protection obtained is antibody mediated. Antibodies from sheep protected by the vaccine can inhibit the protease activity of both H11 and H-gal-GP *in vitro* (Newton & Meussen 2003; Ekoja & Smith 2010; Le Jambre *et al*. 2008) and it is assumed that this also happens *in vivo*.

The concept of vaccination with proteins derived from gut membrane has also proved to be effective in the control of haematophagous arthropods such as ticks. A critical internal component of the parasite, such as a molecule on the gut cell surface, is first isolated and then used to vaccinate the host. In an ectoparasite feeding on blood or tissue fluid and wound exudate, subsequent uptake of blood or other fluid containing antibodies from the host that is accompanied by, for example complement and cells, can lead to immunological damage to the host. This approach is the basis of the *B. microplus* vaccine (Willadsen 1999).

Following vaccination, an antibody response involving high-titre circulation is raised. Antibodies are ingested with the blood during the feeding of the helminths and bind to functional proteins on the brush border of intestinal cells, which compromises digestion. This eventually leads to starvation, loss of fecundity and weakness. Finally, the helminths detach and are expelled (Smith & Zarlega 2006).

Vaccine-induced immunity can be passively transferred by serum or colostrum (Smith 1993, Andrews *et al.* 1997). It has been shown that serum antibody titres are highly correlated with protection (Le Jambre *et al*. 2008).

2.4 Adjuvant

To confer protective immunity, antigens derived from gut membrane have to be administered with an adjuvant. Freund's complete and incomplete adjuvants were widely used. However, in more recent trials QuilA®, a saponin was the adjuvant of choice.

Vaccination trials using Freund's adjuvants have been carried out in the United Kingdom, South Africa and Australia and have involved different sheep breeds, age groups, husbandry systems and challenge regimens with susceptible and anthelmintic-resistant *H. contortus* strains. The mean protection levels achieved were 72% for male worms and 82% for female worms, and a 91% reduction of FECs (Smith *et al* 1999; Newton & Munn 1999). Higher levels of protection have been obtained with QuilA™ as an adjuvant (Newton & Munn 1999).

2.5 Specificity of the vaccine

This *H. contortus* antigen vaccine is genus specific. It is ineffective against *Teladorsagia circumcincta, Trichostrongylus axei* and *Cooperia oncophora* (Smith *et al.* 2001), but protects calves against *H. placei* (Basseto *et al*. 2011).

The antigens H11 and H-gal-GP of *H.contortus*, derived from gut membrane, appear to be highly conserved. Antigens harvested from *H.contortus* in any part of the world are globally effective against *H. contortus* challenge (Smith *et al*. 2001; Munn *et al*. 1993; Souza *et al*. 2011).

An efficacy trial proved the efficacy of the H-gal-GP antigen complex against the anthelmintic-resistant South African White River strain of *H. contortus* (Smith 2007; Smith & Smith 1993).

2.6 Attempts to synthesise recombinant H11 and H-gal-GP antigens

Recombinant versions of H11, H-gal-GP and other antigens did not meet the expectations since they did not confer the adequate degree of protection achieved with the native antigens (Newton & Meeusen 2003; Cachat *et al*. 2010).

2.7 DNA vaccine

The vaccination of goats with DNA vaccines encoding the H11 antigen and IL-2 provided only partial protection against *H. contortus*, resulting in a reduction of faecal egg counts and abomasal worm burdens of 56.6% and 46.7% respectively (Zhoa *et al.* 2012).

As a result of the abovementioned findings, the Moredun Research Institute turned their focus back to the discovered native antigens for the development of a vaccine against haemonchosis of small stock.

Chapter 3
MATERIALS AND METHODS

3.1 Experimental animals

The experimental animals in this trial were 40 clinically healthy three-month-old weaned Dorper lambs that had minimum exposure to worms. All the animals were identified individually.

3.2 Experimental design

The 40 experimental animals were randomly allocated to one of four treatment groups (Groups I, II, III and IV) of 10 animals each. All the animals were weighed and individual faecal egg counts were done. They were then dewormed using a product containing levamisole (Tramisol®). The efficacy of the dewormer was tested by repeating the faecal egg counts. If the faecal egg counts were still positive following treatment with Tramisol®, Unidose®, a product containing trichlorfon, was administered. The purpose of this treatment programme was to obtain gastrointestinal nematode-free sheep for the trial.

3.2.1 *Experimental vaccine*

The immunogen of the experimental vaccine (Wirevax®) was the conconavalin A-binding fraction of the *H. contortus* integral membrane proteins, which consists mainly of H-gal-GP and H11 at a concentration of 5 µg/ml. Quil A was incorporated as an adjuvant. The immunogen and adjuvant were dissolved in tris-buffered saline. Vaccine was formulated, manufactured and made available by Moredun Research Institute.

3.2.2 *Vaccination protocol*

Sheep in Groups I and III were vaccinated by injecting 1 ml of Wirevax® subcutaneously on Days 1, 21 and 42 (Table 3.1).

Sheep in Groups II and IV were untreated controls.

3.2.3 *Artificial worm infection protocol*

The animals were artificially infected with infective *H. contortus* larvae of a non-resistant strain that originated at the Onderstepoort Veterinary Institute of the ARC. The technique

used for infection was that described by Bosvet SOP No NEMATL3INF BV01. All the sheep in Groups I and II were infected orally with 5000 *H. contortus* infective larvae on Day 42, and those in Groups III and IV were infected orally with 5000 *H. contortus* infective larvae on Day 70.

3.2.4 Faecal egg counts

Individual faecal egg counts were conducted on all sheep involved in the vaccine trial and started 16 days after infection, and subsequently twice weekly until the end of the trial, which was on Day 56 for Groups I and II and Day 86 for Groups III and IV. The McMaster method was used to obtain faecal egg counts and the standard operating procedure (SOP) No *EPGMcM BV06* of Bosvet Clinical Development was followed.

3.2.5 Weighing

Vaccinated sheep (Groups I and III) were weighed prior to vaccination and again on 21, 42 and 70 days after vaccination. Those in the control groups (Groups II and IV) were weighed on the same days.

3.2.6 Serology

Serum obtained from the blood samples of vaccinated sheep (Groups I and III) and control groups (Groups II and IV) was screened for seroconversion by means of ELISA. Blood samples collected from the jugular veins of the sheep into EDTA blood-collection tubes were obtained on 1, 21, 42, 49 and 70 days of vaccination. In order to determine the vaccine-antibody titres by ELISA, the SOP of the Moredun Research Institute (*Nematode antigen ELISA SOP 2009*) was followed (Cachat *et al*. 2010).). Briefly, Microlon 96K microtitre plates (Greiner-Bio-One, Frickenhausen, Germany) are coated with 50 µl of a 1 µg ⁄mL antigen solution in carbonate buffer (50 mM carbonate, pH 9.6) and left overnight at 4˚C. Wells were blocked for 2 h at room temperature with 10% (w ⁄ v) Infasoy (Cow & Gate Ltd., Trowbridge, Wiltshire, UK) in TNTT (10 mM Tris-HCl, 0.5 M NaCl, 0.05% Tween 20, 0.01% w ⁄ v thiomersal, pH 7.4). Serum from each animal was diluted to 1: 100 and from that in a series of doubling dilutions to make up 1:200 to 1:204 800 in TNTT; 50 µl was added per well and the plate was incubated for 1 h at room temperature. Mouse monoclonal

anti-goat / sheep IgG-horse radish peroxidase (HRPO) conjugate (50 µl; Sigma), diluted 1:10 000 in TNTT, was added and incubated for 1 h at room temperature. Sigma-Fast OPD substrate was added (50 µl) and incubated at room temperature in the dark for 20 min. The reaction was stopped by the addition of 25 µl 2.5 M sulphuric acid. Absorbance values were read at 490 nm using an ELISA plate reader (Cachat *et al*. 2010, Moredun Research institute Nematode antigen ELISA SOP 2009).

3.2.7 Worm counts

The sheep in Groups I and II were slaughtered on Day 77, 35 days after the *H.contortus* challenge. Groups III and IV were slaughtered on Day 105, 35 days after the *H.contortus* challenge and four weeks after the third vaccination had been given.

Abomasa and their contents were collected at slaughter and processed in the laboratory to quantify the worm burdens based on the recovery of mucosal and luminal stages of *H. contortus.* Published standard procedures were followed for recovery and identification (Capitini *et al*. 1990; Gutierres 1971; Hinaidy *et al.* 1979).

3.2.8 *Data collection and records*

All experimental data for each animal were entered onto prescribed forms used for the capturing of raw data. Subsequently the data were entered on Excel spread sheets.

3.2.9 *Data analysis*

The means, variances and standard errors of the faecal egg counts, post mortem worm counts and mean antibody titres were calculated. The F-test and the Student's T-test (two-tailed test) were used for the statistical analysis of the data. Assessment of efficacy was done according to the following formula:

$$100 - \frac{Mean\ worm\ egg\ count\ in\ treatment\ group}{Mean\ worm\ egg\ count\ in\ control\ group} * 100$$

Faecal egg counts and mean antibody titres were used to construct graphs to indicate significant differences between the vaccinated and control trial groups.

Table 3.1: Vaccine trial schedule

DAY [d]	DATE	ACTIVITY	TRIAL GROUPS
d 1	29/8/2011	First vaccination with Wirevax® Collection of blood samples for serology	I, III I, II, III, IV
d 21	19/09/2011	Second vaccination with Wirevax® Weighing of sheep Collection of blood samples for serology	I, III I, II, III, IV I, II, III, IV
d 28	26/09/2011	Collection of blood samples for serology	I, II, III, IV
d 42	10/10/2011	Third vaccination with Wirevax® Weighing of sheep Collection of blood samples for serology Infection of sheep with *H.contortus* larvae	I, III I, II, III, IV I, II, III, IV I,II
d 49	17/10/2011	Collection of blood samples for serology	I, II, III, IV
d 60	28/10/2011	Faecal egg counts	I, II
d 67	04/11/2011	Faecal egg counts	I, II
d 70	07/11/2011	Weighing of sheep Collection of blood samples for serology Infection of sheep with *H.contortus* larvae	I, II, III, IV I, II, III, IV III, IV
d 74	11/11/2011	Faecal egg counts	I, II
d 77	14/11/2011	Slaughter	I, II
d 88	25/11/2011	Faecal egg counts	III, IV
d 95	02/12/2011	Faecal egg counts	III, IV
d 102	09/12/2011	Faecal egg counts	III, IV
d 105	12/12/2011	Slaughter	III, IV

Chapter 4
RESULTS

4.1 Faecal egg counts

The means of the faecal egg counts of Groups I and II were 210 [epg] and 4760 [epg] respectively. The standard errors of the average faecal egg counts were 116.75 for Group I and 650.98 for Group II. The variances for the average faecal egg counts were 136308.64 for Group I and 4237728.40 for Group II.

The F-test was performed on Group I and Group II sample variances to determine whether the corresponding Group I and Group II population variances, of which these were estimates, were similar at the 5% level of significance for this test. Should the probability demonstrated with the F-test be less than 5%, the assumption of equal population variances can be rejected with at least 95% confidence. From here onward this test procedure was adopted whenever the F-test was applied. The outcome of the F-test is that the probability of the (Group I and Group II) population variances being equal is 0.000020147 (0.002015%). It can therefore be assumed, with at least 95% confidence that the population variances are significantly different. Therefore the unequal variance version of the Student T-test is used to test the assumption of equal population means. When applied to the corresponding means, the probability that the population means will be found to be equal is 0.000053256 (0.005326%), which is much less than 5%. The assumption of equal population means for Groups I and II is rejected with at least 95% confidence (Table 4.1).

The statistical analysis followed to compare Groups I and II, as well as Groups III and IV, population means where the test for equal population variance is rejected, as in this case, was the unequal variance version of the Student T-test. Where the test for equal population variance was not rejected, the equal variance version of the Student T-test will be used.

These results suggest that sheep infected at the third vaccination had decreased faecal egg counts when compared to the control group (Figure 4.1).

The efficacy of vaccination was expressed by percentage protection, which was calculated to a mean of 95.59%, indicating that the contamination of pastures with *H. contortus* eggs is 95.59% less in the case of vaccinated sheep.

The same statistical analyses were conducted on Groups III and IV. The mean faecal egg count for Group III was 760 [epg] and for Group IV 3 910 [epg]. The standard error for Group

III was 470.25 and for Group IV 938.89. The variance for the average faecal egg counts of Group III was 2211308.64 and of Group IV 8815074.07.

Regarding the F-test, the above procedure was repeated on the sample variances of Groups III and IV. The probability of population variances being equal is 0.051539397 (5.15%), which is marginally higher than 5%. Therefore, the equal-variance and unequal-variance Student T-tests were used to test the assumption of equal population means as defined in the test procedure above. The result for the equal-variance student T-test was 0.007688557 (0.77%), whereas for the unequal-variance Student T-test it was 0.010060364 (1.01%). The assumption of equal population means for Groups III and IV is rejected with at least 95% (in fact with 99.23% or 98.99%) confidence (Table 4.2). These results suggest that vaccination resulted in lower faecal egg counts when compared to the control group, even though worm infection occurred 4 weeks after the third vaccination in Groups III and IV (Figure 4.2).

The efficacy of vaccination was expressed by a percentage protection. It calculated to a mean of 80.6% protection, which indicated that the contamination of the pasture with *H.contortus* eggs by vaccinated sheep would be 80.6% less.

Figures 4.1 and 4.2 clearly show that in the case of the control sheep, worm eggs were first detected in the faeces 18 days after the challenge worm infestation was given. On d 25 post challenge, group mean values peaked at about 7 840 [epg] and remained above 3 960 [epg] for the rest of the trial.

In contrast, the mean faecal egg counts for the vaccinated sheep were much lower throughout the trial, peaking at 350 [epg] on Day 25 and remaining below 350 [epg] for the rest of the trial.

4.2 Body weights

The mean body weight gain for Group I was 4.75 [kg] and for Group II 0.3 [kg]. The variances for body weight gain were 7.40 [kg] for Group I and 11.90 [kg] for Group II.

The outcome of the F-test indicated that the probability of the (Groups I and II) population variances being equal was 0.4905 (49.05%), which is well above 5%. It can therefore be assumed that the population variances are not significantly different. The equal variance version of the Student T-test indicated that the probability of the population means to be equal is 0.004930 (0.49), which is well below 5%. The assumption of equal population means for Groups I and II is rejected with at least 95% (actually 99.951%) confidence (Table 4.3).

The same statistical analysis was conducted on Groups III and IV. The mean body weight gain for Group III was 1.85 [kg], while for Group IV it was 4.4 [kg]. The variances for body weight gain were 2.45 for Group III and 14.43 for Group IV. The F-test result was 0.0144 (1.44%), well below 5%. Since it could therefore be assumed, with 95% (actually 98.56%) confidence, that the population variances were significantly different, the unequal variance version of the Student T-test was performed. The result of the calculation was 0.0734 (7.34%), which is above 5%. The assumption of equal population means for Groups III and IV is therefore not rejected (Table 4.4).

A comparison was drawn between Groups I and II and Groups III and IV. The Student T-test results indicated that weight gains in Groups I and II respectively were statistically significant, but that they were not statistically significant for Groups III and IV. No definite statistical correlation between body weight gain and vaccination could therefore be demonstrated.

4.3 Serology

The variance for mean antibody titres were calculated for Groups I and II. The results were 14244832.96 for Group I, and 322421.31 for Group II. The outcome of the F-test was 0.000773155 (0.077%), well below 5%. It can therefore be assumed, with at least 95% (in fact 99.923%) confidence, that the population variances are significantly different. The unequal variance version of the Student T-test was used, as defined in the test procedure discussed in section 4.1. If this is applied to the sample means, the probability that the sample means are equal is 0.0427 (4.27%), which is below 5%. The assumption of equal population means for Groups I and II was rejected with 95% (actually 95.73%) confidence (Table 4.5; Figure 4.3).

The same statistical analysis was conducted on Groups III and IV and the variance for mean antibody titres was calculated for Groups III and IV. The results were 11777614.60 and 416645.60 for the respective groups. The outcome of the F-test was 0.002259 (0.226%), which is well below 5%. It can therefore be assumed with at least 95% (in fact 99.774%) confidence that the population variances differed significantly. The unequal variance version of the Student T-test, as described in section 4.1, was used. The result of the Student T-test was 0.0222 (2.22%), which was well below 5%. The assumption of equal population means

for Groups III and IV was rejected with at least 95% (in fact 97.78%) confidence (Table 4.6; Figure 4.4).

The above results indicate that the mean antibody titres were significantly higher in the vaccinated groups than in the control groups.

An increase in the mean anti-vaccine antibody titres was observed in the vaccinated group at the end of the first week after the second vaccination. Titres then declined over the next seven weeks up to one week after the third vaccination, when they spiked substantially. In contrast, control titres remained at their initial values throughout the trial.

At the commencement of this study antibody titres in both the vaccinated and unvaccinated animals were very low (Figure 4.5).

The first immunisation stimulated a clear anamnestic serological response in the vaccinated animals and two further boosts three weeks apart ensured that high antibody titres were maintained. Each booster vaccination resulted in a spike of antibody production, which tailed off until the next dose was administered.

Even under challenge, the levels of antibodies remained low in the control group from Week 6 to Week 12 of the study. Although antibody concentrations did increase in the control lambs over the course of the trial, they did not come close to the levels of concentration found in the vaccinated animals (Figure 4.5).

The results indicated that the vaccine antigens produced a high level of immune response that was mediated through mechanisms involving antibodies.

4.4 Worm counts

The mean worm counts in sheep of Groups I and II were 240 and 1833 respectively. The variances for Group I were 38 537.16, and for Group II 65663.56.

The result of the F-test was 0.44 (43.94%), which was well above 5%. Since it could thus not be assumed that the sample variances were significantly different, the equal-variance version of the Student T-test was performed. When applied to the corresponding sample means, the probability that the population means would be equal was 0.00000000000661 (0.00000000066%), which is well below 5%. The assumption of equal population means for Groups I and II was rejected with at least 95% (in fact 99.999999934%) confidence (Table 4.7).

The same statistical analyses were conducted on Groups III and IV. The mean worm counts for Group III were 659 and for Group IV 3 406. The variances for Groups III and IV were 432 086.68 and 1617 730.32 respectively.

The outcome of the F-test was 0.0623 (6.23%), which was above 5%. It could therefore not be assumed that the population variances were significantly different. The equal-variance version of the Student T-test, indicated that the probability of the sample means to be equal was 0.0000098 (0.00098%), which is well below 5%. The assumption of equal population means for Groups III and IV was rejected with at least 95% confidence (Table 4.8).

These results suggest that vaccination significantly reduces worm burdens in sheep 35 days post infection. The efficacy of vaccination was indicated by percentage protection. It calculated to a mean of 86.93% protection for Group I, and a mean of 80.6% protection for Group III. *H.contortus* worm counts were shown to be between 80.60% and 86.93% less in vaccinated sheep.

Table 4.1: Faecal egg counts of sheep in trial groups I and II

	Post challenge egg counts					
		2011/10/28	2011/11/04	2011/11/11	Average post challenge	% Protection eggs
Group 1	**Animal no**	**EPG**	**EPG**	**EPG**	**EPG**	
(Vaccinated)	V1	0.00	0.00	0.00	0.00	100.00
	V16	0.00	2200.00	1000.00	1066.67	77.59
	V2	0.00	0.00	0.00	0.00	100.00
	V3	0.00	0.00	0.00	0.00	100.00
	V4	0.00	1100.00	1000.00	700.00	85.29
	V33	0.00	100.00	300.00	133.33	97.20
	V14	0.00	0.00	0.00	0.00	100.00
	V27	0.00	0.00	200.00	66.67	98.60
	V30		0.00	0.00	0.00	100.00
	V49	0.00	100.00	300.00	133.33	97.20
	Mean	**0**	**350.00**	**280**	**210**	**95.59**
	se	0.00	232.02	126.32	116.75	2.45
	Variance	-	538 333.33	159 555.56	136 308.64	60.16
Group 2	V29	3200	12000	8000	7733.33	
(Control)	V8	1600	12000	8400	7333.33	
	V36	2200	2800	2100	2366.67	
	V42	300	10000	5600	5300.00	
	V32	4200	16000	1200	7133.33	
	V38	3000	3800	1300	2700.00	
	V17	2600	5400	5400	4466.67	
	V31	2000	6200	2800	3666.67	
	V45	1900	4200	1200	2433.33	
	V21	3800	6000	3600	4466.67	
	Mean	**2480.00**	**7840.00**	**3960.00**	**4760.00**	
	se	360.19	1385.90	869.51	650.98	
	Variance	1 297 333.33	19 207 111.11	7 560 444.44	4 237 728.40	

Assumption test	**Test**	**Probability**	**Population conclusions for Group I and II**	**Student T-test version to use:**
Population variances equal	**F-test**	0.000020147	Variances not equal	Unequal variances
Population means equal	**Student T-Test**	0.000053256	Means not equal	

Table 4.2: Faecal egg counts of sheep in trial groups III and VI

	Post challenge egg counts					
		2011/11/25	2011/12/02	2011/12/09	Average post challenge	% Protection eggs
Group 3	**Animal no**	**EPG**	**EPG**	**EPG**		
(Vaccinated)	V18	700	3400	4500	2866.67	26.68
	V41	0	400	200	200.00	94.88
	V20	0	0	100	33.33	99.15
	V12	0	0	0	0.00	100.00
	V13	0	0	0	0.00	100.00
	V37	200	100	300	200.00	94.88
	V50	0	0	400	133.33	96.59
	V48	100	6200	6200	4166.67	-6.56
	V39	0	0	0	0.00	100.00
	V44	0	0	0	0.00	100.00
	mean	**100.00**	**1010.00**	**1170.00**	**760.00**	**80.6**
	se	69.92	666.41	709.47	470.25	12.03
	Variance	48 888.89	4 441 000.00	5 033 444.44	2 211 308.64	1 446.42
Group 4	V22	0	12200	8400	6866.67	
(Control)	V6	2800	9000	7100	6300.00	
	V24	0	700	2400	1033.33	
	V5	200	3200	7800	3733.33	
	V11	700	1200	1700	1200.00	
	V19	200	1200	3400	1600.00	
	V47	2200	16600	11400	10066.67	
	V34	300	3800	5100	3066.67	
	V35	200	3100	7500	3600.00	
	V46	200	1600	3100	1633.33	
	mean	**680.00**	**5260.00**	**5790.00**	**3910.00**	
	se	312.62	1728.66	992.24	938.89	
	Variance	977 333.33	29 882 666.67	9 845 444.44	8 815 074.07	

Assumption test	**Test**	**Probability**	**Population conclusions for Group III and IV**	**Student T-test version to use:**
Population variances equal	**F-test**	0.051539397	Variances not equal	Unequal variances and Equal variances
Population means equal	**Equal Student T-test**	0.007688557	Means not equal	
Population means equal	**Unequal Student T-Test**	0.010060364	Means not equal	

Table 4.3: Body weights of sheep in trial groups I and II

Animal weight record						
	Treatment	Date: 22/8/2011	Date: 19/9/2011	Date: 10/10/2011	Date: 7/11/2011	Weight
Animal Number	Group	Kg	Kg	Kg	Kg	Gain/(Loss)
V2	I	23.5	20	23.5	24.5	1.0
V16	I	27	25.5	28.5	30	3.0
V14	I	28.5	25	29	31	2.5
V1	I	28.5	28.5	33.5	36.5	8.0
V4	I	29.5	30	33	35	5.5
V33	I	31	30	33	36.5	5.5
V3	I	31.5	30.5	31.5	34.5	3.0
V27	I	32	29	33	35.5	3.5
V30	I	33	37	39	43	10.0
V49	I	34	33.5	38	39.5	5.5
	Total	298.5			346.00	47.5
	Mean	29.85			34.60	4.75
	Variance	9.78			26.54	7.40
V29	II	25.5	26.5	26	27.5	2
V8	II	27	26	28.5	29.5	2.5
V36	II	28	26.5	28.5	30	2
V42	II	28	21.5	22	25.5	-2.5
V32	II	29	23.5	23.5	22.5	-6.5
V38	II	30	27.5	31.5	32	2
V17	II	31	25	27	27	-4
V31	II	32	32.5	37.5	36	4
V45	II	33	30.5	32	33.5	0.5
V21	II	33.5	30	34.5	36.5	3
	Total	297			300.00	3.00
	Mean	29.7			30.00	0.30
	Variance	7.07			20.72	11.90

Assumption test	**Test**	**Probability**	**Population conclusions for Group I and II**	**Student T-test version to use:**
Population variances equal	**F-test**	0.4905	Variances equal	Equal variances
Population means equal	**Student T-Test**	0.004930	Means not equal	

Table 4.4: Body weights of sheep in trial groups III and IV

Animal weight record						
	Treatment	Date: 22/8/2011	Date: 19/9/2011	Date: 10/10/2011	Date: 7/11/2011	Weight
Animal Number	Group	Kg	Kg	Kg	Kg	Gain/(Loss)
V18	III	25.5	21.5	24.5	28	2.5
V41	III	27.5	22	28.5	28.5	1
V20	III	28.5	25.5	29.5	31	2.5
V12	III	29	28.5	30.5	31	2
V13	III	30	29	31.5	31.5	1.5
V37	III	30.5	29.5	31.5	31.5	1
V50	III	31.5	30	33.5	33.5	2
V48	III	32	28	34	33	1
V39	III	33.5	30.5	34.5	39	5.5
V44	III	33.5	29.5	31.5	33	-0.5
	Total	301.5			320	18.5
	Mean	30.15			32	1.85
	Variance	6.73			9.33	2.45
V22	IV	26.5	29	34	35	8.5
V6	IV	26.5	26.5	30.5	30	3.5
V24	IV	27.5	24	26.5	25	-2.5
V5	IV	28.5	29	25	34.5	6
V11	IV	29.5	30	35	33.5	4
V19	IV	30.5	25	31	32.5	2
V47	IV	31.5	29	33	32	0.5
V34	IV	32.5	35	41	42.5	10
V35	IV	32.5	32.5	39	40	7.5
V46	IV	33.5	33	38.5	38	4.5
	Total	299			343	44
	Mean	29.9			34.3	4.4
	Variance	6.71			25.34	14.43

Assumption test	**Test**	**Probability**	**Population conclusions for Group III and IV**	**Student T-test version to use:**
Population variances equal	**F-test**	0.0144	Variances not equal	Unequal variances
Population means equal	**Student T-Test**	0.0734	Means equal	

Table 4.5: Mean antibody titres of sheep in trial groups I and II

Group mean antibody titres

	Vaccination 1	Vaccination 2		Vaccination 3			
	2011/08/29	2011/09/19	2011/09/26	2011/10/10	2011/10/17	2011/11/07	**Variance**
Group I (Vaccinated)	593	2204	7889	6942	10235	8278	14 244 831.96
Group II (Control)	828	1923	2257	2366	2175	1682	322 421.31

Assumption test	Test	Probability	Population conclusions for Group I and II	Student T-test version to use:
Population variances equal	**F-Test**	0.00077315	Variances not equal	Unequal variances
Population means equal	**Student T-test**	0.0427	Means not equal	

Table 4.6: Mean antibody titres of sheep in trials groups III and IV

Group mean antibody titres

	Vaccination 1	Vaccination 2		Vaccination 3			
Date	2011/08/29	2011/09/19	2011/09/26	2011/10/10	2011/10/17	2011/11/07	**Variance**
Group III (Vaccinated)	1 200	2 399	7 265	6 984	9 865	8 319	11 777 614.60
Group IV (Control)	737	1 814	2 413	1 475	1 616	751	416 645.60

Assumption test	Test	Probability	Population conclusions for Group III and IV	Student T-test version to use:
Population variances equal	**F-Test**	0.002259	Variances not equal	Unequal variances
Population means equal	**Student T-test**	0.0222	Means not equal	

Table 4.7: Number of worms recovered from sheep in trial groups I and II

Postmortem worm counts			
	Animal no	**H.c Adult**	**H.c L4**
GROUP I	V1	220	-
VACCINATED	V2	77	-
	V3	255	-
	V4	669	-
	V14	195	-
	V16	450	-
	V27	196	-
	V30	268	-
	V33	10	-
	V49	56	-
	TOTAL	2 396	-
	MEAN	240	-
	VARIANCE	38 537.16	-
	% EFFECTIVE	**86.93**	
GROUP II	V8	1 518	-
CONTROL	V17	2 113	-
	V21	1 639	-
	V29	2 005	-
	V31	2 112	-
	V32	2 029	-
	V36	1 377	-
	V38	1 740	-
	V42	1 850	-
	V45	1 947	-
	TOTAL	18 330	-
	MEAN	1 833.00	-
	VARIANCE	65 663.56	-

Assumption test	Test	Probability	Population conclusions for Groups I and II	Student T-test version to use:
Population variances equal	**F-test**	0.44	Variances equal	Equal variances
Population means equal	**Student T-Test**	0.00000000000661	Means not equal	

Table 4.8: Number of worms recovered from sheep in trial groups III and IV

Postmortem worm counts			
	Animal no	**H.c Adult**	**H.c L4**
GROUP III	V50	300	-
VACCINATED	V48	1 306	-
	V41	616	-
	V20	379	-
	V18	2 143	-
	V13	877	-
	V12	85	-
	V37	726	-
	V39	55	-
	V44	100	-
	TOTAL	6 587	-
	MEAN	659	-
	VARIANCE	432 086.68	-
	% EFFECTIVE	**80.66**	
GROUP IV	V5	3 838	-
CONTROL	V6	3 773	-
	V11	2 225	-
	V19	1 408	-
	V22	4 039	-
	V24	1 560	-
	V34	4 440	-
	V35	4 860	-
	V46	3 169	-
	V47	4 749	-
	TOTAL	34 061	-
	MEAN	3 406	-
	VARIANCE	1 617 730.32	-

Assumption test	Test	Probability	Population conclusions for Groups I and II	Student T-test version to use:
Population variances equal	**F-test**	0.0623	Variances equal	Equal variances
Population means equal	**Student T-Test**	0.0000098	Means not equal	

Figure 4.1: Mean faecal egg counts and efficacy percentage in sheep in trial groups I and II

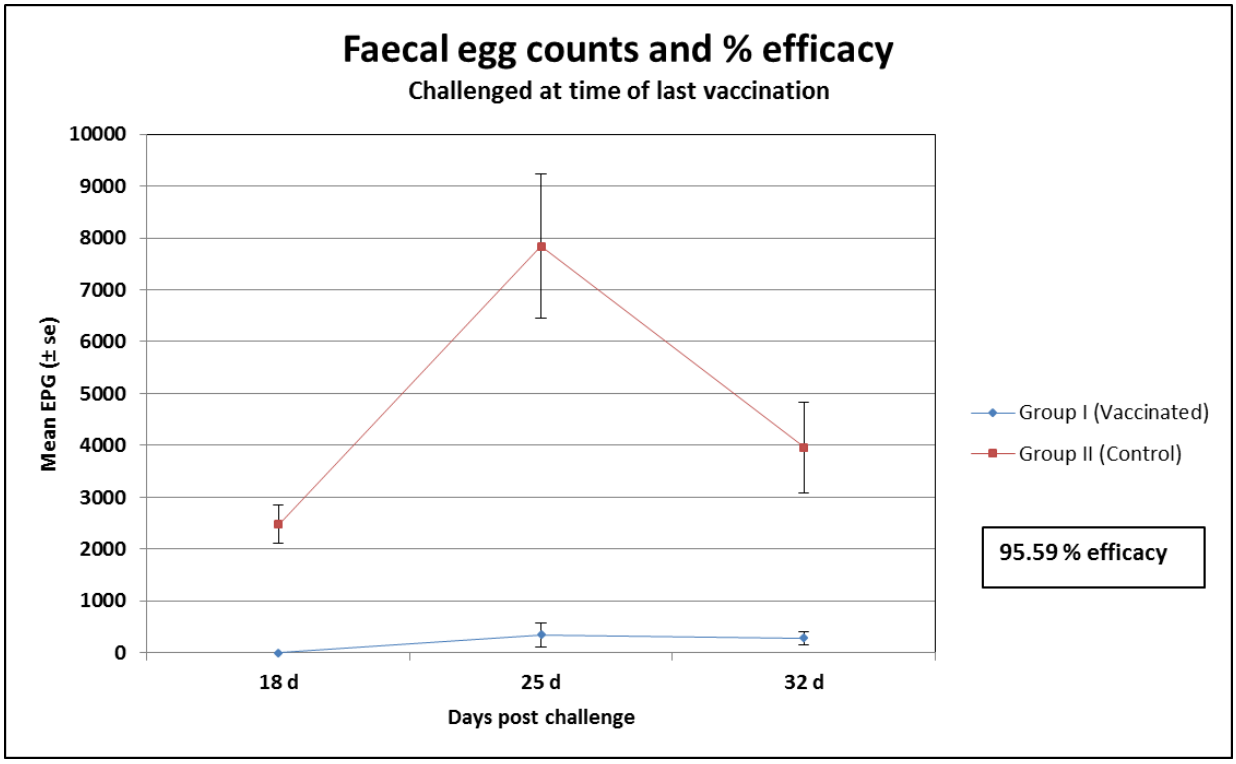


Figure 4.2: Mean faecal egg counts and efficacy percentage in sheep of trial groups III and IV

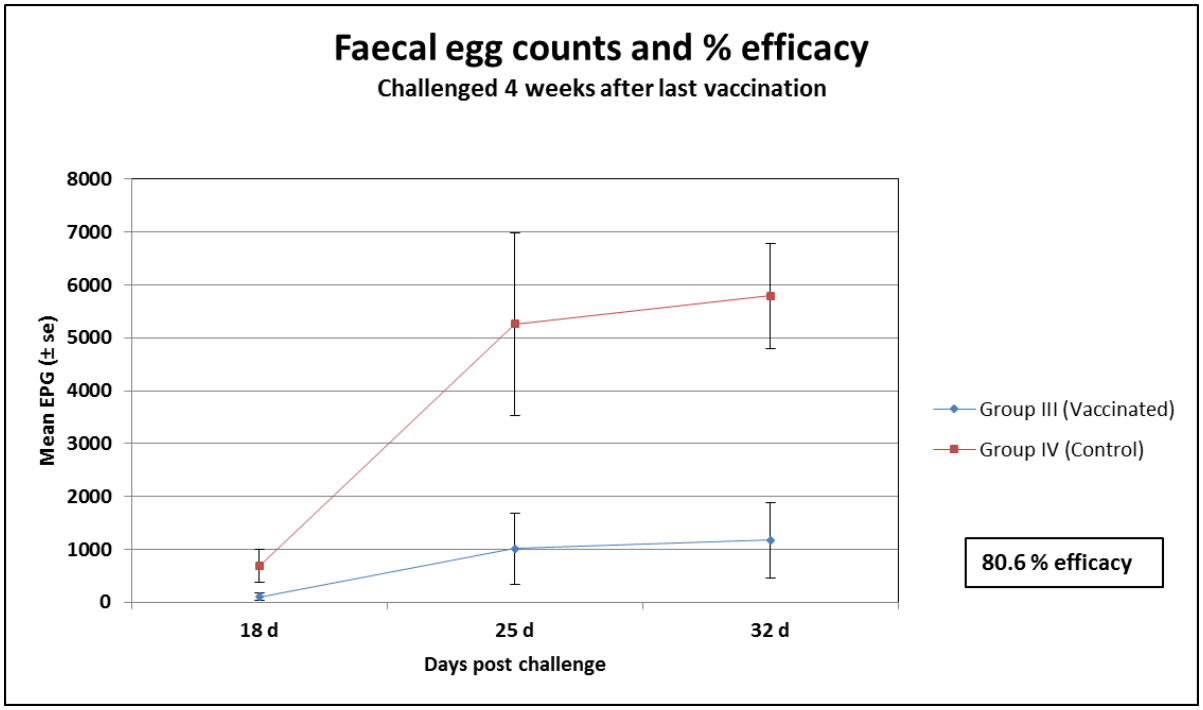


Figure 4.3: Group mean antibody titres of sheep in trial groups I and II

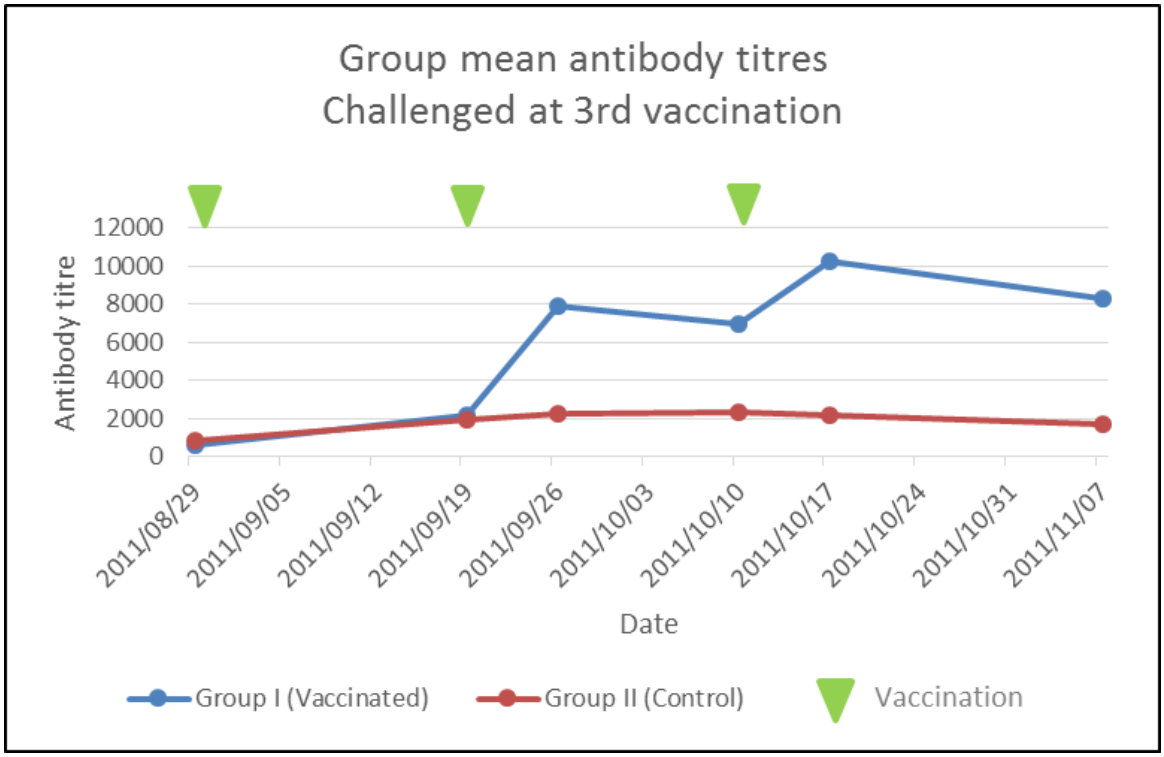


Figure 4.4: Group mean antibody titres of sheep in trial groups III and IV

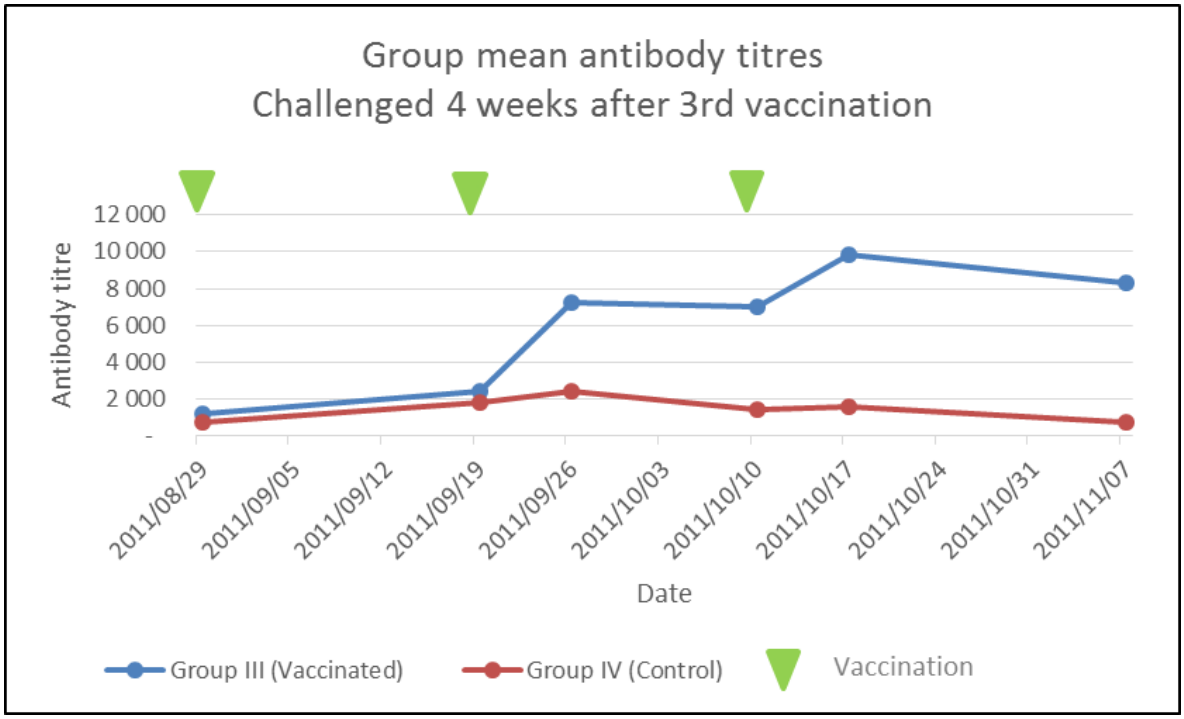
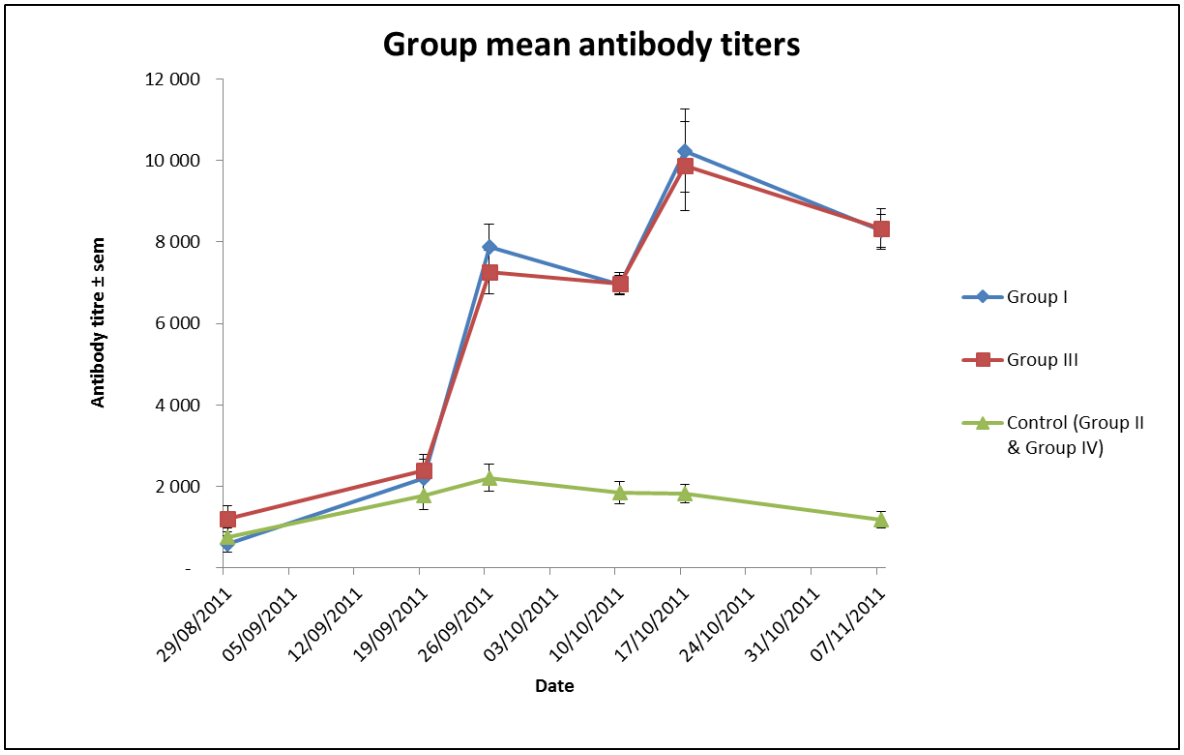


Figure 4.5: Comparative group mean antibody titres of sheep in trial Groups I, II, III, IV



Chapter 5
DISCUSSION

This study was conducted to confirm the efficacy of the experimental vaccine Wirevax® in South Africa with the aim to obtain product registration as the first step to commercialise this product in South Africa.

Effective vaccination would be an alternative control measure to address haemonchosis in small stock and to limit the reliance on anthelmintics. The additional benefit would be to slow down the development of anthelmintic resistance and to enable the small stock farming industry in South Africa to remain productive and competitive.

The results of the trial indicated that a strong protective immunity develops in vaccinated sheep. After three vaccinations, given three weeks apart, the experimental vaccine Wirevax® stimulated a strong circulating antibody response. Statistically, the mean antibody titres of the vaccinated sheep were significantly higher than those measured in the controls. This immune response was associated with reductions in faecal egg counts and worm burdens in vaccinated sheep compared to control sheep. In sheep challenged immediately after the third vaccination, the efficacy based on worm counts was 86.93% and on faecal egg counts were reduced by 95.59%. In those sheep that were challenged 30 days after the third vaccination, the efficacy was 80.66 % on worm counts and faecal egg counts were reduced by 80.6%. It can be concluded that three vaccinations given at three-week intervals will aid in the control of haemonchosis in sheep and will significantly limit new infections for 30 days.

Barnes *et al.* (1995) developed a mathematical model for simulating *Trichostrongylus* populations in grazing sheep and compared the use of theoretical vaccines of nominal efficacy with the use of conventional control methods based on anthelmintic treatment. It was concluded that vaccines based on hidden antigens conferred a protection of 80% of the flock with 80% efficacy would ensure better control than a conventional anthelmintic programme.

The results of this study correlate with findings of other studies using this antigen vaccine combination (H-gal-GP and H11) (Le Jambre *et al*. 2008). Field studies conducted over 11 months under natural grazing were conducted in South Africa and gave similar protection levels to what was found here (Smith *et al.* 2001). It is recommended that field studies be

conducted under South African conditions, using sheep known to be infected with multiple resistant *H.contortus* strains.

It is recommended that vaccination starts 9 weeks prior to the *Haemonchus* season. Three injections 3 to 4 weeks apart are needed to induce protection (80% of the flock with 80% efficacy as stated above), after that, immunity can be maintained by boosters given at 6 week intervals until the first frost lowers infection rate.

Wirevax® is now registered in Australia and marketed commercially as Barbervax®.

Chapter 6
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